Chemical Disinfection and Corrosion Prevention

By H. L. A. Tarr
Pacific Fisheries Experimental Station
Vancouver, B.C.

(*Received for publication August 6, 1946*)

ABSTRACT

The effectiveness of solutions of disinfectants in killing bacterial vegetative cells in a watery suspension of fish slime, on dry artificially contaminated wooden surfaces and in nutrient broth, as well as their ability to destroy bacterial endospores and mould spores, was investigated. The disinfectants studied included sodium hypochlorite, chloramine T, six cationic detergents, four chloro- or phenyl-phenols, phenol, formaldehyde, a commercial cresolic disinfectant and another containing sodium hypochlorite. The use of sodium nitrite as a corrosion inhibitor for three of the disinfectants when in contact with iron and copper was also investigated. The bearing of the results on the selection of disinfectants for use in the fishing industry is discussed.

There is still a pressing need for general improvement in the market quality of edible fish products, and one means of bringing this about is by adequate disinfection of fishing vessels, fish handling plants and retail premises.

In 1927 Bronkhorst (pp. 88-89), in connection with experiments on the control of bacterial reddening of salted fish, suggested that chemical disinfectants be used both on fishing vessels and in buildings in which the fish was prepared. He advised a thorough washing of all surfaces, preferably with hot water, and subsequent treatment with a chemical bactericide such as 2% sulphurous acid, "formalin" or potassium permanganate. He also believed that boats, and garments which workers used in handling the fish, should be sterilized. Later Bedford (1935) advocated that fishing vessels, after thorough cleansing with water, be disinfected by means of a 6% formalin solution applied with a high pressure spray. Gibbons (1935) stated that plants preparing salted fish should employ germicidal sprays such as a 2% formalin or "lye" solution in order to help control the red discolouration of the product. Hess (1940) gave general instructions for disinfection of plants which handle fish, and suggested that rooms might be sealed and sterilized by use of sulphur dioxide gas or formalin vapour, or that they could be sprayed with 2.5% formalin or lye solution. Hypochlorites have also been used quite extensively as disinfectants in the fishing industry. Dybwad (1937) recommended their use in canneries.

About two years ago experiments were undertaken at this Station in order to study a number of disinfectants, certain of which it was thought might prove suitable for use in the fishing industry, and the results of this work are described

J. Fish. Res. Bd. Can. 7 (3) 1947.
Printed in Canada.

herewith. Brief preliminary accounts of this investigation have already been published (Tarr 1944, 1945).

Since Shewan (1945) found that dehydrated fish contain coccoid organisms potentially capable of producing food-poisoning, a known enterotoxin-producing coccus strain (*Staphylococcus aureus*) was included in the experiments.

EXPERIMENTAL

Disinfectants

1. Sodium hypochlorite. One hundred and ten g. of H.T.H. powder (Mathieson), containing about 70% of calcium hypochlorite, and 60 g. of anhydrous sodium carbonate were dissolved separately in distilled water. The solutions were mixed, diluted to 1 litre, and filtered. The filtrate contained about 8% of sodium hypochlorite, had a pH of approximately 11.2 and was stable for months when stored in the dark at O°C.

2. Diversol. An alkaline powder containing 3.5% of sodium hypochlorite.

3. Roccal and R2L. Ten per cent solutions of alkyl dimethyl benzyl ammonium chloride.

4. Thoral. Ten per cent solution of 9-octadecenyl dimethyl ethyl ammonium bromide.

5. Cetavlon. A powder containing 75% of cetyl trimethyl ammonium bromide.

6. Emulsept and Norcalite. Ten per cent solutions of N-(acyl esters of colamino formyl methyl) pyridinium chloride.

7. E 607 M. The N-(myristic acid ester of colamino formyl methyl) pyridinium chloride.

(Nos. 3 to 7 inclusive cationic detergents.)

8. Chloramine T. Eastman Kodak Co.

9. Lysol. Lehn and Fink.

10. Dowicide A. Sodium ortho phenylphenate (Anon. 1941).

11. Dowicide C. Sodium chloro-2-phenylphenate (Anon. 1941).

12. Dowicide D. Sodium 2-chloro-4-phenylphenate (Anon. 1941).

13. Dowicide F. Sodium 2, 3, 4, 6-tetrochlorophenate (Anon. 1941).

14. Phenol. Merck's U.S.P.

15. Formaldehyde. Baker's C.P.

Cultures

1. *Staphylococcus aureus*. ("Wood" strain, enterotoxin producer, from the University of British Columbia.)

2. *Micrococcus* sp. Culture 6, isolated from fish (Tarr 1939).

3. *Achromobacter* sp. Culture 9, isolated from fish (Tarr 1939).

4. *Achromobacter* sp. Culture 17, isolated from fish (Tarr 1939).

5. *Achromobacter* sp. Culture 22, isolated from fish (Tarr 1939).

6. *Bacillus* sp. Unclassified aerobic sporogenous *Bacillus* isolated from soil.

7. *Penicillium cyclopium* (from the National Regional Research Laboratory, Peoria, Ill.).

8. *Penicillium puberulum* (from the National Regional Research Laboratory, Peoria, Ill.).

PREPARATION OF BACTERIAL SUSPENSIONS

Fish Slime Suspension

A suspension containing a mixture of fish spoilage bacteria in a watery extract of fish slime was prepared as follows. A number of lemon soles (*Parophrys vetulus*) and herring (*Clupea pallasii*), which had been stored for a few days in ice, were washed in several litres of tap water so that a fairly cloudy suspension was obtained, from which the coarser particles were removed by filtering with suction through glass wool. The resulting suspension was stored for 1 day at about 5°C., after which it had a direct count (Tarr 1943) of 85×10^6 bacteria per ml. and a viable count (Tarr and Bailey 1939) of 4.1×10^6 colonies per ml. The apparent discrepancy in these counts was probably due to the fact that there were numerous clumps of bacteria in the suspension. A number of ¼-lb. (113-g.) tins were filled with the suspension, sealed, frozen in still air and stored at between about −25° and −30°C. until required. The frozen suspension was thawed immediately prior to use by immersing a can in water at 20 to 25°C. The following viable counts were obtained during the interval of storage throughout which the suspension was employed in experiments to be described: 3 days' storage, 740,000 colonies per ml.; 67 days' storage, 210,000 colonies per ml. The suspension contained 11.3 mg. of dry material in 5 ml.

Mixed Bacterial Vegetative Cells

Ten 9-cm. diameter petri dishes containing Bacto nutrient agar, pH 7, were inoculated with each of cultures 2, 3, 4 and 5 and were incubated for 3 days at 25°C. The growth was suspended in 2 l. of sterile distilled water and filtered with suction through sterile absorbent cotton wool. The suspension was then frozen and stored as in the case of suspension 1, and the following counts were obtained:

	Direct count, bacteria per ml. (Petroff-Hausser counting chamber)	Viable count, colonies per ml. (Tarr & Bailey 1939)
Unfrozen, immediately prior to freezing..	$7{,}000 \times 10^6$	$9{,}100 \times 10^6$
Thawed, after 1 day's storage.....	$6{,}500 \times 10^6$	$8{,}700 \times 10^6$
Thawed, after 7 days' storage.....	—	$7{,}900 \times 10^6$
Thawed, after 373 days' storage.....	$7{,}000 \times 10^6$	$4{,}450 \times 10^6$

The suspension was used for disinfection experiments within 40 days of preparation.

Endospores

A number of petri dishes containing a 1.5% agar medium prepared from a 1:10 dilution of fish digest broth at pH 7.0 (Tarr 1942) as nutrient substrate were inoculated with culture 6 and incubated for 7 days at 30°C. The growth, which consisted mainly of endospores on this dilute medium, was suspended in sterile distilled water, filtered through absorbent cotton wool with suction and heated for 10 minutes at 80°C. The resulting suspension was frozen and stored in the usual manner, and the following counts were obtained:

	Direct count (spores per ml.)	Viable count (colonies per ml.)
Unfrozen, immediately prior to freezing..	290×10^6	110×10^6
Thawed, after 2 days' storage.....	330×10^6	120×10^6
Thawed, after 333 days' storage.....	280×10^6	85×10^6

The suspension was used between 300 and 330 days after preparation.

Mould Spores

Cultures 7 and 8 were grown for 14 days at 25°C. on each of ten 9-cm. petri dishes containing Bacto nutrient agar to which 5% glucose and 0.5% tartaric acid had been added (Henrici 1930). The mould spores were suspended in sterile distilled water, the suspension was filtered with suction through cotton wool and centrifuged, the precipitated spores were re-suspended in water, filtered again and frozen and stored in the usual manner. The following counts were obtained:

	Direct count (spores per ml.)	Viable count (colonies per ml.)
Immediately prior to freezing.....	48×10^6	29×10^6
Thawed, after 1 day's storage.....	49×10^6	27×10^6
Thawed, after 21 days' storage.....	49×10^6	21×10^6

In the experiments to be described, sterile tubes, pipettes and dilution water were used, and aseptic precautions were taken in making all dilutions of the germicides.

RESISTANCE TO DISINFECTANTS

Bacteria in Fish Slime

The relative effectiveness of the different germicides in presence of fish slime was determined as follows. Five-millilitre portions of appropriate serial dilutions of the germicide in 19 × 150-mm. tubes were placed in a water bath at 20°C., and a 5-ml. portion of thawed fish slime suspension (20°C.) was added to each tube and mixed thoroughly. After 10 minutes a 4-mm. loop was inserted into each tube and was withdrawn so that only a thin film of liquid adhered to it. This was transferred to a tube containing 10 ml. of Bacto nutrient broth (pH 7.0). Tubes thus inoculated were incubated for 7 days at 25°C. and the presence or

absence of growth was recorded. The final dilutions of germicide used in making these tests were as follows: 1:20 to 1:100 (intervals of 1:20), 1:100 to 1:1000 (intervals of 1:100), 1:1000 to 1:18,000 (intervals of 1:1000). In the results (table I), it will be seen that, under the conditions given, the cationic detergent germicides, with the exception of the one which was contaminated, were active in low concentrations. From about 0.02 to 0.1% of a germicide of this type would rapidly kill fish spoilage bacteria in the presence of a moderate amount of fish slime. Sodium hypochlorite and chloramine T showed about the same degree of germicidal activity as did the cationic detergents. The disinfecting power of Diversol corresponded very closely to its sodium hypochlorite content. The Dowicides and Lysol were somewhat less effective than the cationic detergents and hypochlorite, but were considerably better germicides than phenol or formaldehyde.

TABLE I. Comparative effectiveness of disinfectants against the natural mixed bacterial population of fish slime.

Disinfectant	Lowest concentration of disinfectant which prevented growth in sub-cultures
Roccal	1:10,000 / 1: 9,000 } 1:9,500
R 2 L	1:8,000 / 1:7,000 } 1:7,500
Norcalite	1:6,000
Thoral	1:5,000
Cetavlon	1:5,000
E 607 M	1:4,000
Emulsept*	All tubes down to 1:16,000 dilution showed growth.
Sodium hypochlorite	1: 9,000 / 1:10,000 } 1:9,500
Chloramine T	1:5,000
Diversol	1:300
Dowicide A	1:600
" C	1:2,000
" D	1:2,000
Lysol	1:500
Formaldehyde	1:100
Phenol	1:100

*Two different samples were examined and both were found to be contaminated with a spore-forming *Bacillus*. Viable counts showed that one sample contained 1.7×10^6 and the other about 20 colonies per ml. of disinfectant. This contamination did not seriously impair the activity of this germicide toward certain bacteria, as is shown by experiments on disinfection of surfaces, which will be described.

ENDOSPORES

The technique was similar to that employed in the above experiment, except that thawed endospore suspension was employed in place of fish slime suspension, and a full 4-mm. loopful of medicated suspension was transferred to nutrient

broth as in the phenol coefficient test (Ruehl and Brewer 1931). The experiments extended over a 24-hour period. Two separate experiments were made; in one, undiluted endospore suspension was employed, and in the other the suspension was diluted 1:10 with water before use. The results (table II) show that, of the six disinfectants tested, only sodium hypochlorite rapidly killed the endospores. Formaldehyde killed the spores very slowly, and phenol and Dowicide D were inactive even after 24 hours. In 0.05% concentration Roccal and Norcalite did not inactivate the spores within 24 hours. The results with 0.5% of these compounds were shown in other experiments to be unreliable owing to the fact that usually sufficient germicide to prevent growth was carried over into the broth tubes used for sub-cultures. Very little difference was observed in the behaviour of the undiluted and diluted spore suspension under the conditions of the experiment.

TABLE II. Effect of disinfectants on bacterial endospores from culture 6. A = undiluted spore suspension; B = spore suspension diluted 1:10; + = visible growth; − = no visible growth.

Germicide	Concentration %	Exposure time (20°C.)																					
		5 min.		10 min.		20 min.		30 min.		1 hr.		2 hr.		3 hr.		4 hr.		5 hr.		6 hr.		24 hr.	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Phenol	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Roccal	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Norcalite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	−	−	−	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dowicide D	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Formaldehyde	0.5	+	+	+	+,	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sodium hypochlorite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

MOULD SPORES

The experimental conditions were similar to those used for bacterial endospores, except that thawed undiluted and diluted (1:10) mould spore suspension was tested, transfers being made into Bacto nutrient broth containing 5% glucose and 0.5% tartaric acid. The results (table III) show that sodium hypochlorite killed mould spores more rapidly than did the other compounds tested, and that it was closely followed in effectiveness by Roccal and Thoral. Norcalite,

Dowicide D and formaldehyde killed mould spores rather slowly. The results obtained in the case of 0.5% Dowicide D were not very reliable, because tests indicated that a quantity of this disinfectant sufficient to inhibit growth of mould in the broth used for sub-culture was carried over by the 4-mm. loop employed.

TABLE III. Effect of disinfectants on mixed mould spores from cultures 7 and 8. A = undiluted spore suspension; B = spore suspension diluted 1:10; + = excellent growth with surface mycelial "mat"; +* = slight growth consisting of a clump of mycelia in the liquid and no surface growth; − = no growth.

Germicide	Concentration %	Exposure time (20°C.)																					
		5 min.		10 min.		20 min.		30 min.		1 hr.		2 hr.		3 hr.		4 hr.		5 hr.		6 hr.		24 hr.	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Phenol	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Roccal	0.5	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+*	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Norcalite	0.5	+	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	−	+	−	−	−	−	−	−	−
	0.05	+*	+*	+*	+*	+*	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Dowicide D	0.5	−	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+*	+	+	+	+	+	+	+	+	+	+	+*	+	+	+*	+*	+*	−	−	−	−	−
Formaldehyde	0.5	+	+	+	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*	+	+*	+	+*	−	−
Sodium hypochlorite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thoral	0.5		+*		−		−		−		−		−		−		−		−		−		−
	0.05		+*		+*		−		−		−		−		−		−		−		−		−

EFFECT OF ANTI-CORROSIVE

Roccal and formaldehyde were selected for this experiment because the former was shown to be very effective in killing mixed fish spoilage bacteria in the presence of fish slime, and because the latter has been used extensively as a germicide in the fishing industry. Sodium hypochlorite was not tested in this way because attempts to find a really satisfactory anti-corrosive for this compound have not as yet yielded very favourable results (*vide infra*). Phenol, Lysol and the Dowicides were not studied because they possess a distinct and rather penetrating odour which, although not detracting from their usefulness for certain disinfecting purposes, makes them appear rather unsuitable for use near fish or fish products. Sodium nitrite was selected for use as anti-corrosive for reasons which will be given later.

Cultures 1 and 5 were used as test organisms, and the standard F.D.A. phenol coefficient method (Ruehle and Brewer 1931) was followed, with the single exception that all cultures and subcultures were grown in Bacto nutrient broth (pH 7.0). Culture 1 was grown at 37° and culture 5 at 25°, the phenol coefficient being determined at 20°C. in the case of each culture. The results (table IV) show that sodium nitrite tends to increase the germicidal efficiency of both formaldehyde and Roccal, and that this is true for solutions in which nitrite and germicide were stored together for a considerable time. They also show that Roccal is a very much more effective germicide than is formaldehyde.

TABLE IV. Influence of sodium nitrite on the germicidal efficiency (on cultures 1 and 5) of Roccal and formaldehyde as judged by the phenol coefficient test.

Method of treatment of the germicide from which dilutions were made to determine the phenol coefficient.	Phenol coefficient	
	Culture 1	Culture 2
Untreated formaldehyde	1.6	2.0
5% formaldehyde containing 1% sodium nitrite used directly after preparation	1.6	2.4
5% formaldehyde containing 1% sodium nitrite stored 57 days in a closed clear glass bottle at room temperature	1.6	—
Untreated Roccal	235	110
Solution containing 1% each of Roccal and sodium nitrite stored 120 days in a closed brown glass bottle at room temperature	294	116

CONTAMINATED SURFACES

The method to be described was used in order to obtain some information regarding the ability of different concentrations of the germicides being studied to inactivate bacteria present on dry, heavily contaminated wooden surfaces.

Five 6.25-cm.^{2} areas were ruled at 2.5-cm. intervals on the smooth planed surfaces of a number of 25 × 6.25 × 2-cm. fir boards. Prior to use, and between experiments, these were washed with hot trisodium phosphate solution, next with water, then with dilute acetic acid and again with water. They were sterilized by autoclaving and permitted to dry at room temperature. The boards were then placed on a level surface and 5 ml. of thawed mixed bacterial cell suspension were painted along a strip about 4 cm. wide so that all ruled areas on each surface were covered. The suspension was dried at room temperature with assistance of a fan. From 2 to 3 hours after contamination the surfaces were gently immersed for 5 seconds in a 200-ml. portion of either water or appropriately diluted germicide contained in a shallow glass dish. After immersion the boards were placed vertically and exposed to a fan so that the surfaces dried in about 5 minutes. The surfaces of the 5 ruled squares on each board were then washed thoroughly into 9 ml. of sterile water using a small cotton swab on an applicator, a suspension of roughly the same opacity being obtained in each case. The

numbers of viable bacteria in these suspensions were determined using the roll tube method.

The results (table V) will be seen to be very erratic, and this may be due

TABLE V. Decrease of viable bacteria on treating with different disinfectants wood surfaces contaminated with a mixed bacterial suspension.

Disinfectant	Concentration %	Viable bacteria (colonies per ml. of washings)		Reduction by disinfectant in viable bacterial population (%)	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Water (controls)		14×10^6 17×10^6 20×10^6 21×10^6 34×10^6 44×10^6 Average = 25×10^6		—	—
Sodium hypochlorite....	1.0	2,000	9	> 99.99	> 99.99
	0.1	11,000	62,000	> 99.99	99.8
	0.05	40,000	200,000	99.8	99.2
	0.01	370,000	230,000	98.6	98.9
	0.005	450,000	720,000	98.2	97.2
Roccal	1.0	0	0	100	100
	0.1	47,000	83,000	99.8	99.7
	0.05	430,000	1.1×10^6	98.1	95.6
	0.01	7.1×10^6	7.7×10^6	67.6	69.1
	0.005	11×10^6	14×10^6	56.0	44.0
Emulsept.....	1.0	0	0.5	100	> 99.99
	0.1	700,000	1.2×10^6	97.2	95.2
	0.05	7×10^6	1.8×10^6	72.0	92.8
	0.01	32×10^6	8.9×10^6	0	64.4
	0.005	28×10^6	15×10^6	0	40
E 607 M.....	1.0	0	2.0	100	> 99.99
	0.1	620,000	3.4×10^6	97.3	86.4
	0.05	7×10^6	24×10^6	72	4.0
	0.01	15×10^6	—	40	—
	0.005	15×10^6	—	40	—
Cetavlon.....	1.0	1.5	1.0	> 99.99	> 99.99
	0.1	1.3×10^6	2.2×10^6	94.8	91.2
	0.05	3.2×10^6	12×10^6	87.2	52
	0.01	9.4×10^6	31×10^6	62.4	0
	0.005	10×10^6	14×10^6		

Disinfectant	Concentration %	Viable bacteria (colonies per ml. of washings)		Reduction by disinfectant in viable bacterial population (%)	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Dowicide A.....	1.0	47,000	28,000	99.8	99.8
	0.1	14×10^6	27×10^6	44	0
	0.05	42×10^6	23×10^6	0	8
	0.01	35×10^6	—	0	—
	0.005	20×10^6	—	20	—
Dowicide C.....	1.0	74	3,000	> 99.9	> 99.9
	0.1	550,000	630,000	98.0	97.3
	0.05	4.2×10^6	3.9×10^6	83.0	84.4
	0.01	11×10^6	—	56.0	—
	0.005	20×10^6	—	20.0	—
Dowicide D.....	1.0	0	0	100	100
	0.1	1.2×10^6	3.1×10^6	95.2	87.6
	0.05	16×10^6	17×10^6	36.0	32.0
	0.01	17×10^6	—	32.0	—
	0.005	25×10^6	—	0	—
Dowicide F.....	1.0	19,000	3,500	> 99.9	> 99.9
	0.1	20×10^6	19×10^6	20.0	24.0
	0.05	17×10^6	29×10^6	32.0	0
	0.01	26×10^6	—	0	—
	0.005	24×10^6	—	4	—
Formaldehyde...	1.0	130	650	> 99.9	> 99.9
	0.1	3.3×10^6	17×10^6	86.9	32
	0.05	9.8×10^6	35×10^6	60.8	0
	0.01	20×10^6	44×10^6	20.0	0
	0.005	22×10^6	34×10^6	12.0	0
Phenol.....	1.0	147,000	200,000	99.6	99.2
	0.1	7.7×10^6	40×10^6	69.1	0
	0.05	14×10^6	13×10^6	44.0	48.0
	0.01	13×10^6	—	48.0	—
	0.005	16×10^6	—	36.0	—
Lysol.....	1.0	4,500	1,100	> 99.9	> 99.9
	0.1	5×10^6	16×10^6	80.0	36.0
	0.05	7.1×10^6	35×10^6	71.6	0
	0.01	14×10^6	—	44.0	—
	0.005	27×10^6	—	0	—
Diversol.....	1.0	11,000	27,000	> 99.9	> 99.9
	0.1	920,000	1.5×10^6	96.3	94.0
	0.05	970,000	2.6×10^6	96.1	89.6
	0.01	3.6×10^6	6.0×10^6	85.6	76.0
	0.005	7.9×10^6	11×10^6	68.4	56.0

to a number of factors, such as uneven contact of germicide and bacteria on the surfaces, differences in time of contact between germicide and bacteria resulting from differences in rate of drying, variations in intensity of bacterial suspension obtained on swabbing the surfaces, variations in time from the making of the bacterial suspension to the completion of the viable counts during which the organisms were in some cases exposed to the germicide, and finally to a possible inhibitory effect of the germicide if carried over into the lower dilutions used in making the viable counts. The results can, therefore, give only a very rough idea of the effectiveness of the germicides in sterilizing surfaces. If an arbitrary standard of 99 to 100% kill be considered excellent, 90-99% good, and less than 90% relatively ineffective, then the following conclusions can be drawn.

Excellent: Sodium hypochlorite, 1.0, 0.1 and 0.05%; Roccal, 1.0 and 0.1%; 1% of all the other disinfectants studied.

Good: Sodium hypochlorite, 0.01 and 0.005%; Roccal, 0.05%; Emulsept, 0.1% (0.05% in one of two tests); E 607 M, 0.1% (in one of two tests); Cetavlon, 0.1%; Dowicide C, 0.1% (in one of two tests); Dowicide D, 0.1% (in one of two tests) and Diversol, 0.1 and 0.05%.

Relatively ineffective: 0.1% or less of Dowicide A, Dowicide F, formaldehyde, phenol and Lysol.

In general these results are rather similar to those obtained in the experiment using fish slime suspension, where sodium hypochlorite and Roccal appeared to be the most effective of the disinfectants studied, the other cationic detergents also proving very active. In both types of experiment, Dowicide A, formaldehyde and phenol were relatively ineffective.

PREVENTION OF CORROSION

On fishing boats and in fish plants, corrosion of metals, particularly iron, steel, copper and brass, must be controlled. Certain disinfectants are particularly corrosive (e.g. hypochlorites), while others are not. However, solutions of the latter type will cause corrosion of metal surfaces to the same extent as will water itself. For this reason a means of neutralizing the corrosive effect of watery solutions of disinfectants was sought. A number of inorganic salts such as chromates, hexametaphosphates and nitrites have been used to inhibit corrosion of certain metals. Of these, sodium nitrite was selected because its weak solutions are practically colourless and almost neutral. It has been used extensively as an anti-corrosive in alkaline washing powders used for cleaning surgical instruments and, more recently, for preventing corrosion in oil pipe lines (Wachter and Smith 1943) and of steel in water (Wachter 1945).

At first a corrosion inhibitor was sought for use with sodium hypochlorite solutions, since this disinfectant was shown by the above experiments to be the best all-round germicide of those studied. Tests showed that nitrite could not be used for this purpose, since it reacts with hypochlorite. Sodium hexametaphosphate (Calgon) and potassium chromate appeared to afford slight protection of iron surfaces in presence of hypochlorite, but neither could be considered as

really satisfactory. As a result of these findings, and for reasons already given in the section in which the effect of nitrite on the phenol coefficients of Roccal and formaldehyde was described, corrosion prevention was studied only in the case of three of the disinfectants, namely Roccal, Emulsept and formaldehyde.

The following qualitative method was used to determine the relative rate of corrosion of iron and copper in solutions of the disinfectants. Squares of polished black iron or copper with a surface area of 6.5-cm.^{2} and a thickness of approximately 0.05 cm. were placed in 9-cm.-diameter open petri dishes. Twenty millilitres of distilled water or germicide solution of the desired strength (with or without sodium nitrite as anti-corrosive) were added to each dish. The dishes were stored at room temperature, 20 ml. of water being added to each dish every 4 days to make up for evaporation in cases where the test extended over 4 days. Corrosion was determined qualitatively by recording the amount of visible rusting of iron, or green discolouration of copper, in the experimental solutions. The results (tables VI and VII) show that the slight corrosion of

TABLE VI. Prevention of corrosion of black iron in formaldehyde solutions. The signs − to ++++ indicate relative degrees of corrosion of the metals.

Disinfectant	% $NaNO_2$ present	*Comparative corrosion after 4 days
2.5% formaldehyde (unbuffered).....	0	++
	0.01	−
	0.05	−
	0.1	−
	0.5	−
*2.5% formaldehyde (buffered).....	0.0	++++
	0.01	++++
	0.05	−
	0.1	−
	0.5	−
	1.0	−

*The unbuffered formaldehyde had a slightly acid reaction. The buffered solutions of formaldehyde were slightly alkaline in reaction, and were prepared from 5% formaldehyde solution containing 0.2% $Na_2HPO_4.12H_2O$.

black iron which occurs in formaldehyde solutions is entirely prevented by quite low concentrations of sodium nitrite. They also show that slightly alkaline solutions of formaldehyde corrode iron more readily than do untreated formaldehyde solutions which are faintly acidic in reaction, and that alkaline solutions require slightly higher concentrations of nitrite to prevent such corrosion. The experiments with Roccal indicated that its watery solutions are not appreciably more corrosive toward black iron than is water alone, and that the corrosion of iron which does occur in Roccal solutions can be very markedly inhibited by sodium nitrite when it is present in a concentration similar to that of the detergent itself. The anti-corrosive action of nitrite was more marked in

the case of Emulsept solutions than with Roccal. Noticeable inhibition of corrosion of copper was only evident in solutions containing 0.1 or 1.0% of detergent and sodium nitrite, and was more marked with Emulsept than with Roccal.

TABLE VII. Prevention of corrosion of black iron and copper in solutions of Roccal and Emulsept. The signs − to ++++ indicate relative degrees of corrosion of the metals.

Disinfectant %	$NaNO_2$ %	Comparative corrosion after days:					
		Iron			Copper		
		4	16	40	4	16	40
Roccal							
0	0	++++	++++	++++	−	+	+
0	0.01	−	−	−	+	+	+
0.01	0	++++	++++	++++	+	++	++
0.01	0.01	−	+	++++	+	++	++
0.1	0	++++	++++	++++	++	++	++
0.1	0.01	++	++++	++++	++	++	++
0.1	0.1	−	+	+	+	+	++
1.0	0	++++	++++	++++	+++	++++	++++
1.0	0.01	++++	++++	++++	+++	++++	++++
1.0	1.0	−	+	+	++	++++	++++
Emulsept							
0.01	0	+++	++++	++++	−	+	+
0.01	0.01	−	−	++++	−	+	+
0.1	0	++++	++++	++++	+	+	+
0.1	0.01	−	+++	++++	+	+	+
0.1	0.1	−	−	−	−	+	+
1.0	0	++++	++++	++++	++	++	++++
1.0	0.01	++++	++++	++++	++	++++	++++
1.0	1.0	−	−	−	−	+	+

DISCUSSION

Though the experiments which have been described above do not definitely show that any one of the disinfectants studied is invariably superior to another for use in the fishing industry, they do give a definite indication of those which may prove of value. Sodium hypochlorite was undoubtedly the best all-round disinfectant, because it not only destroyed bacterial vegetative cells very readily but also destroyed both bacterial endospores and mould spores. However, it is very corrosive toward iron, copper and, indeed, many other metals (Prucha 1930), and if employed should be washed away soon after use as Moulton (1929) suggests. The cationic detergents as a group killed bacterial vegetative cells when used in quite low concentrations, and certain of them also killed mould spores fairly rapidly, but they were practically ineffective against bacterial

endospores. Tests with two of these detergents showed that sodium nitrite could be added to their watery solutions as an anti-corrosive, and that in the case of one of them the added nitrite did not adversely affect its germicidal potency. For these reasons it would appear that cationic detergents offer definite possibilities as disinfectants for use in the fishing industry. The fact that they were not effective in killing bacterial endospores would not seriously detract from their value, because endospore-forming *Bacilli* are not very frequently encountered among the common fish spoilage bacteria, and the presence of a cationic detergent, with the possible exception of the samples of Emulsept used which contained viable endospores (table I), would undoubtedly inhibit the germination of the spores and the growth of the resulting vegetative cells. Numerous references have appeared since 1935 in which preparation, properties, germicidal power and methods of analysis of cationic detergents have been described (Domagk 1935, 1938; Maier 1939; Krog and Marshall 1940; Baker, Harrison and Miller 1941; Auerbach 1943; Epstein, Harris and Katzman 1943; Epstein, Harris, Katzman and Epstein 1943; Hoogerheide 1945; Valko and Dubois 1945). It is unlikely that the Dowicides would prove of value for purposes of general disinfection because, although those studied proved quite good germicides, all possessed a rather penetrating odour which would render them somewhat unsuitable for use near fish or fish products. This is also true of Lysol and phenol. Formaldehyde was shown to be one of the least effective of the germicides studied, and although it has been used extensively in the fishing industry, and is compatible with sodium nitrite as anti-corrosive, its low potency would detract considerably from its germicidal value. Formaldehyde solutions are also unpleasant to use. Diversol, which is apparently considerably less corrosive than sodium hypochlorite (Prucha 1930), is a good disinfectant, its effectiveness being roughly proportional to its sodium hypochlorite content.

ACKNOWLEDGMENTS

I wish to thank Canadian Industries Ltd., The Emulsol Corporation, The Diversey Corporation, Winthrop Chemical Co., G. H. Wood and Co., The Turco Corporation and Merck and Co. for supplying certain of the disinfectants used in this work. To Mrs. F. M. Kwong I am indebted for technical assistance.

SUMMARY

The effectiveness of a number of disinfectants in inactivating bacterial vegetative cells, bacterial endospores and mould spores was investigated. The following types of disinfectants were studied: sodium hypochlorite, cationic detergents, phenyl- or chloro-phenols, a cresol compound, phenol and formaldehyde. The methods of testing the disinfectants included treatment of mixed fish spoilage bacteria in the presence of a watery suspension of fish slime, disinfection of mixed dried bacterial cells on wood surfaces, employment of a standard phenol coefficient method and medication of aqueous suspensions of bacterial endospores or mould spores. The possibility of preventing the corrosion of iron and copper which might arise through use of disinfectants was also studied.

The results indicated that sodium hypochlorite was the best all-round disinfectant, since it killed bacterial vegetative cells very readily, and also killed mould spores and bacterial endospores. Diversol exerted a germicidal activity which was closely related to its sodium hypochlorite content. Attempts to prevent corrosion, which is marked in the presence of hypochlorite, were not successful. The cationic detergents destroyed bacterial vegetative cells quite readily, and two of them inactivated mould spores rapidly. None of those tested was an efficient killer of bacterial endospores. In the case of two of the cationic detergents tested, sodium nitrite was found to inhibit more or less strongly corrosion of iron and, to a smaller extent, of copper. In the case of one of these germicides the addition of sodium nitrite was found to have no adverse effect on its phenol coefficient. Though the phenolic germicides were quite effective bactericides, their solutions possess a marked odour and it is not known whether this would detract from their practical value. Both phenol and formaldehyde were poor disinfectants. The slight corrosion of iron which occurred in watery solutions of formaldehyde was abolished by addition of a small amount of sodium nitrite.

REFERENCES

ANON. Dowicides, industrial germicides and fungicides, 1-44, Dow Chem. Co., Midland, Mich., 1941.

AUERBACH, M. E. *Ind. Eng. Chem.*, **15**, 492-493, 1943.

BAKER, Z., R. W. HARRISON AND B. F. MILLER. *J. Exper. Med.*, **74**, 611-620, 1941.

BEDFORD, R. H. *Bull. Fish. Res. Bd. Can.*, **49**, 1-8, 1935.

BRONKHORST, M. *France Off. Sci. tech. Pêches Mar. Notes et Rapp.*, **53**, 1-168, 1927.

DYBWAD, P. *Pac. Fisher.*, **35** (5), 25-26, 1937.

DOMAGK, G. *Deut. med. Wochschr.*, **61**, 829-832, 1935. (*Chem. Abst.* **29**, 701) B. U.S. Patent No. 2,108,765, 1938.

EPSTEIN, A. K., B. R. HARRIS AND M. KATZMAN. *Proc. Soc. Exper. Biol. Med.* **53**, 238-241, 1943.

EPSTEIN, A. K., B. R. HARRIS, M. KATZMAN AND S. EPSTEIN. *Oil and Soap*, **20**, 171-174, 1943.

GIBBONS, N. E. *Fish. Res. Bd. Can. Prog. Rep. Atl.*, **14**, 13-14, 1935.

HENRICI, A. T. Moulds, yeasts and actinomycetes, 1-296, Wiley, New York, 1930.

HESS, E. *Fish. Res. Bd. Can. Prog. Rep. Atl.*, **27**, 3-5, 1940.

HOOGERHEIDE, J. C. *J. Bact.*, **49**, 277-289, 1945.

KROG, A. J., AND C. G. MARSHALL. *Amer. J. Pub. Health*, **30**, 341-348, 1940.

MAIER, E. *J. Bact.*, **38**, 33-39, 1939.

MOULTON, C. R. Meat through the microscope, 1-528, University of Chicago Press, 1929.

PRUCHA, J. M. *Milk Dealer*, **19**, 104-110, 1930.

RUEHLE, G. L. A., AND C. M. BREWER. *U.S. Dep. Agri. Cir.* **198**, 1-20, 1931.

SHEWAN, J. M. *J. Hygiene*, **44**, 193-207, 1945.

TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1939.
J. Fish. Res. Bd. Can., **6**, 74-89, 1942.
J. Fish. Res. Bd. Can., **6**, 119-128, 1943.
Fish. Res. Bd. Can. Prog. Rep. Pac., **59**, 7-9, 1944.
Fish. Res. Bd. Can. Prog. Rep. Pac., **62**, 12-13, 1945.

TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.

VALKO, E. I., AND A. S. DUBOIS. *J. Bact.*, **50**, 481-490, 1945.

WACHTER, A. *Ind. Eng. Chem.*, **37**, 749-751, 1945.

WACHTER, A., AND S. SMITH. *Ind. Eng. Chem.*, **35**, 358-367, 1943.

Estimation of Corrosion in Canned Herring

By Andrew Hollett
Fish Inspection Laboratory
Halifax, N.S.

(*Received for publication November 25, 1946*)

ABSTRACT

Metallic odour and flavour of canned herring in plain cans was found to be proportional to the concentration of extractable iron. There was also a rise in pH with increase of corrosion. No relation was found between the tin content and corrosion, metallic odour and flavour being due largely, if not entirely, to the iron dissolved from the steel base plate.

The urgent need for food to meet the requirements of the armed forces and the peoples of Europe provided a tremendous impetus for the production of canned fish in Canada during the second world war. In the Maritime provinces production of canned herring (*Clupea harengus*), in "one pound tall" cans was begun in 1941 and increased tremendously in the years following. Up to and during 1944 a large proportion of the herring was put up in "plain" cans—cans without interior enamel coating. Examination at this Laboratory of herring in plain cans revealed that some few weeks after canning the interior of the cans began to show evidence of corrosion. Some areas were brown or purplish but, for the most part, the discoloured areas were black. At that time the contents were generally normal in odour, flavour and appearance. After continued storage the discoloured areas increased and the steel-grey colour of the base plate became visible. Concomitant with this, there was definite deterioration in the quality of the can contents, the fish often having a definitely metallic odour and flavour. Sometimes the can contents were extremely metallic, even nauseating, and showed greenish blue, blue-black, or even black discolouration. Although the complete history of many cans examined was not known, it may be estimated that extreme corrosion, as indicated above, was evident after storage of a year or more at room temperature. Similar conditions resulted when mackerel (*Scomber scombrus*) was canned in plain cans, but the rate of internal corrosion of the cans was much less rapid. The canning of alewives (*Pomolobus pseudoharengus*) in plain cans also resulted in rapid corrosion of the can interior.

This investigation was primarily an attempt to measure, objectively, metallic odour and flavour (corrosion) of fish put up in plain cans, and to estimate the maximum period the fish could be stored before metallic odour and flavour would render it inedible, without consideration of any physiological effects which tin and iron might have on the consumer, a subject which will be taken up in the

J. Fish. Res. Bd. Can. 7 (3) 1947
Printed in Canada.

discussion. Metallic discolouration of fish accompanied by pronounced metallic odour and flavour, with or without discolouration, can be so objectionable to the prospective consumer as to render such a product inedible.

Spot tests carried out on the interior of many cans in various stages of corrosion indicated that initially the black spots are tin compounds, largely stannous oxide (Brennert 1935). With dissolution of tin in some areas and subsequent exposure of the underlying steel plate, iron sulphide forms. When this occurs, the iron sulphide and oxide, if oxygen is available, are deposited in the fish, giving rise to the discoloured condition noted in a preceding paragraph. Jackson, Howat and Hoar (1936) noted that black patches and black specks in the body of canned cream consisted of ferrous sulphide, stannous oxide or both. Black discolouration of the cream was noted only after the steel plate had been exposed.

In estimating can corrosion, analyses were made to determine the concentration of tin and iron in the canned fish. The pH of the aqueous free liquid in the cans was also estimated, using a Leeds and Northrup glass electrode.

TIN

Tin was estimated colorimetrically by a modification of the silicomolybdate method of Baker, Miller and Gibbs (1944). The complete contents of a can of fish were placed in the disintegrator cup of a Waring Blendor and ground to a homogeneous mass. If there was little free liquid in the can, it was necessary to add 20 to 25 ml. of distilled water to facilitate grinding. When water was added, the can was first rinsed with it to remove adherent particles of fish. The weight of the can contents was determined by weighing the can before and after the removal of the fish, and in the final calculations a factor was applied to correct for the addition of water. Ten-gram samples of ground fish were used for hydrolysis with the sulphuric-hydrochloric acid mixture, and heating in the Claisen flask was continued only until solution of the sample was effected. Following distillation of the tin as stannous bromide by the method outlined by Baker, Miller and Gibbs (1944) the distillate was reduced in volume, transferred to a 25 ml. (or 50 ml.) volumetric flask, made to volume, and aliquots of this solution used for colour development. The volume of the aliquot was varied according to the concentration of tin in the original sample.

It will be seen from table I that there was no apparent relationship between tin concentration and subjective rating for metallic odour and flavour. In some cases, the tin content was low but there was a distinct metallic odour and flavour; in others the odour and flavour were normal but the tin content ranged as high as 245 parts per million. This would indicate that either tin does not contribute to the metallic odour and flavour of fish in corroded cans, or that it plays a relatively unimportant part in comparison with that of iron.

IRON

The ground fish, prepared as directed for tin analyses, was also used for analyses of iron. At the beginning of the investigation, iron dissolved from the steel plate was estimated by deducting the normal concentration of iron in the

TABLE I. Typical analyses of canned herring in plain cans (unless otherwise stated) for iron, tin and pH, showing their relation to degree of corrosion and period of storage.

Approximate storage period	Description	pH	mg. per g. fish		
			Iron		Tin
			Extractable	Total	
7 months	Herring. Few areas of can walls and bottom black, otherwise can interior bright. Not more than trace of metallic odour	6.5	0.005	0.0077	
7 months	Herring. Normal odour, flavour and colour. Few black areas on sides, no apparent detinning. Trace of metallic odour from empty can	6.35	0.0056		0.245
7–8 months	Herring. Odour and colour normal. Few black areas on can, no detinned area	6.35	0.006	0.0096	0.142
8 months	Herring. Non-metallic odour, few black areas on can interior	6.65	0.0075	0.012	
8 months	Herring. Normal odour, few black areas only	6.4	0.0054	0.0135	
8 months	Herring. Slightly metallic, some detinned areas, some black areas, many areas "tin" bright	7.0	0.068	0.097	0.5
10 months	Herring. Very slightly metallic odour. Flavour normal, few black areas on can, few small detinned areas	6.5	0.011		0.14
10 months	Herring. Interior of can mostly gray, few black areas, some greenish discolouration of the meat. Metallic odour	7.1	0.086	0.129	0.477
10 months	Herring. About three-quarters surface grey to black, remainder bright. Odour definitely metallic, some discolouration of meat	7.3	0.115	0.145	0.44
10 months	Herring. Can interior mostly steel grey, few black areas. Some greenish to black discolouration of meat. Strongly metallic odour	7.4	0.20	0.26	0.85
10 months	Herring. Can interior mostly grey, remainder black. Strongly metallic, meat somewhat discoloured near can surface.	7.5	0.28	0.33	0.613
20 months	Herring. Practically entire can interior steel grey or black. Very strong metallic odour. Green to black discolouration of meat	7.95		0.186	1.0
20 months	Herring. Interior badly corroded. Odour very strong, almost nauseating. Meat badly discoloured with greenish-black areas	8.25		0.5	0.68

TABLE I—*Continued*

Approximate storage period	Description	pH	mg. per g. fish		
			Iron		Tin
			Extractable	Total	
3 years?	Herring in tomato sauce. Extremely metallic, green to black discolouration of the meat. Can interior almost completely detinned.....		0.135		0.87
7-8 years	Lobster. C-enamel can. Slightly metallic odour, no apparent corrosion.....		0.042		
10 years	Mackerel. C-enamel cans. Strong odour, somewhat metallic. No visible corrosion.	6.35	0.135	0.14	0.025
?	Herring. Highly metallic, with discolouration of the meat. Steel plate visible in some areas.....	7.5	0.137		
7 months	Herring. C-enamel, no corrosion.....	6.35		0.018	None
8 months	Herring. C-enamel, no corrosion.....	6.4	None	0.016	Too low for estimation
8 months	Herring. C-enamel, no corrosion.....	6.45	0.004	0.019	
20 months	Herring. C-enamel, no corrosion.....		None	0.03	None

fish muscle from the total iron concentration in the can. During the course of the investigation a method was developed for directly determining extractable iron, or the amount of iron dissolved from the steel plate. Iron was estimated, using thioglycollic acid as a reagent. Swank and Mellon (1938) have made a critical study of the effect of the common cations and anions of the thioglycollic acid method for iron estimation and they found this method superior to various other colorimetric procedures. It is particularly suitable for the estimation of iron in biological material, in that there is no interference in colour development by other ions in concentrations in which they normally occur in animal tissues. Modifications of the method outlined by Tompsett (1934) and "The B. D. H. book of organic reagents" (1941) were used.

Total iron was estimated by heating a 10 g. comminuted sample with 10 ml. concentrated sulphuric and 10 ml. concentrated perchloric acid in a 200 ml. Kjeldahl flask. The sample was weighed in a 50 ml. tared beaker and transferred to the flask with the aid of the acid. The mixture was heated, very cautiously at first, then more vigorously, till all the organic matter was destroyed, the perchloric acid driven off, and the solution practically colourless. When the sample contained considerable fat, more rapid hydrolysis was effected by the addition of a few drops of 30% hydrogen peroxide during the latter part of digestion. After hydrolysis the solution was transferred to a 100 ml. volumetric flask, and neutralized with concentrated ammonium hydroxide, using a small piece of litmus paper as indicator. For colour development an aliquot of this solution was transferred to a 50 ml. volumetric flask, and to it were added 1 ml. of 10% thio-

glycollic acid solution neutralized with concentrated ammonium hydroxide, and 5 ml. of 3 N ammonium hydroxide, and the solution made to volume. After about 5 minutes the red colour, which was quite stable, was read in an Evelyn photoelectric colorimeter, using a filter having a maximum transmission at 440 mμ. A null reading was obtained by preparing a solution as outlined above, substituting distilled water for the sample of fish. By using highest quality reagents, a blank was obtained that was colourless to visual observation. The concentration of iron was determined from the formula $C = \frac{2 - \log G}{K}$, where "C" equals concentration of iron, "2 − log G" (per cent light transmission) was obtained from tables, and "K" was previously determined by using solutions containing known amounts of iron.

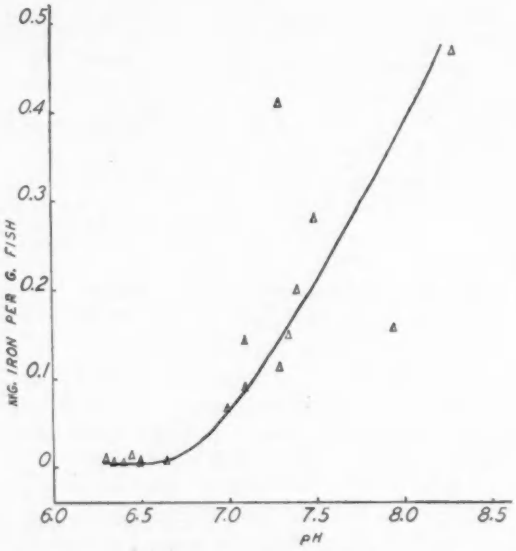

FIGURE 1. Relation between pH of the aqueous liquid and the concentration of extractable iron of herring in plain cans.

Extractable iron was determined by mixing 10 g. of the ground fish with 15 ml. of 10% trichloracetic acid solution and 5 ml. of 10% thioglycollic acid solution. After thorough mixing, it was filtered through a no. 40 Whatman filter and the iron content of the filtrate determined as outlined above, but omitting the thioglycollic acid solution. The volume of filtrate taken for analysis varied with the degree of corrosion. If there was a strong metallic odour from the sample, 1 or 2 ml. of the filtrate were taken for analysis; in the absence of pronounced metallic odour, about 10 ml. were taken. Null readings were determined by preparing solutions from fish from enameled cans showing no evidence of corrosion. Solutions thus prepared were practically colourless. This method

of estimating what, for convenience, is termed "extractable iron" was found very satisfactory. It directly measured the amount of iron dissolved from the steel plate, thus eliminating the necessity of estimating total iron in the can contents with subsequent deduction of the normal iron content. The method was rapid; duplicate analyses could be performed in 20 to 30 minutes.

With increase in degree of metallic odour and flavour, both in canned herring and canned mackerel, there was an increase in the concentration of extractable iron (table II and figure 1). These findings support the contention of Shrader (1939) that metallic taste in canned products is not due to the small amounts of tin, but rather to the iron that has been dissolved. Table I gives typical results of analyses for iron, tin and pH, with descriptive information on the condition of the can interior and the contents.

It was found that the concentration of iron in canned herring in enameled cans showing no evidence of corrosion varied from less than 0.01 to 0.03 mg. per 100 g. fish. Analyses indicated that there was a reciprocal relationship between the fat content and the iron content, the lowest values for iron being present in fish high in fat content.

Dill and Clark (1926) analysed four cans of spider crab and concluded that there was a fair correlation between the amount of iron and the degree of blackening, but that the amount of tin and the amount of blackening were unrelated.

TABLE II. Relation between metallic odour, pH and extractable iron in canned herring (plain cans)

Odour	pH	Extractable iron (mg. per g. fish)
Non-metallic or trace only		Less than 0.015
Slightly metallic	6.6	0.016–0.07
Metallic	6.7–7.2	0.071–0.1
Strongly metallic	7.3–7.6	0.11 –0.3
Extremely metallic	7.6 up	0.31 up

Table II gives a tentative relationship between extractable iron and intensities of metallic odour and flavour. The table is based on organoleptic examination and analyses of the contents of some thirty cans of fish, chiefly herring. It is applicable to both canned herring and canned mackerel, but the figures should be revised upward a little for the latter product.

HYDROGEN ION CONCENTRATION

The hydrogen ion concentration of the aqueous liquid was estimated by the glass electrode using a Leeds and Northrup pH meter. The oil in the free liquid was separated from the aqueous liquid by means of a separatory funnel.

A fairly close correlation between increase in pH and the concentration of extractable iron is shown in figure 1. It would seem that tin compounds play a

relatively unimportant part in increasing the alkalinity. This is shown in table I, in which one can of herring with pH of 6.4 had an extractable iron content of 0.0063 mg. per g. and a tin content of 0.085, and another with pH 6.35 an iron content of 0.0056 and tin content of 0.245. In contrast, a can with pH of contents 7.1 had a concentration of 0.086 for iron, and 0.477 for tin.

The pH of the aqueous liquid from newly canned herring put up from perfectly fresh fish has been found to be 6.3 to 6.4, which is therefore the basic pH. There is what may be considered a normal variation of pH (table I) in commercially canned herring in non-corroded cans, depending to a great extent on the freshness of the fish at the time of canning. Since the pH varies normally from

TABLE III. Corrosion and vacuum in canned herring (plain cans)

Vacuum (in.)	Number of cans			
	No corrosion	Slight corrosion	Corroded	Very corroded
0		4		
1		3		
2	1	6	2	
3		6	1	
4		7	8	
5		5	6	
6		5		
7	2	11	2	
8	4	35	20	
9	6	34	6	2
10	2	23	8	
11	6	12	15	4
12	1	5	11	
13	1	7	5	
14				
15		3		
16 up		2	1	1

6.3 to 6.6 in commercially canned herring depending upon the freshness of the fish at the time of canning, it is evident that very limited corrosion of the can interior will not be recognizable by the pH. With progressive corrosion there is a corresponding rise in pH, the highest value recorded being 8.35 with an extractable iron concentration of 0.48 mg. per g. fish. Dill and Clark (1926) showed that in canned spider crab the pH rose from 7.6 in normal cans to 8.3 in cans showing much blackening.

Jackson *et al.* (1936) made a study of discolouration in canned cream due to corrosion of the can interior, determining the pH, which ranged from 6.58 to 6.93, but apparently making only qualitative tests for iron, tin and sulphide. Examination of their data indicated that, in contrast to our findings, there was no relation among pH, iron, tin and sulphide, or any combination of them.

VACUUM

The assertion by many investigators that oxygen is a limiting or controlling factor in the degree of internal corrosion in canned products (see Discussion) is not supported by the results of the inspection, at this laboratory, of 283 cans of herring in plain cans (table III). If the degree of corrosion were related to the amount of gaseous oxygen available in the can, it would be expected that, presupposing equal headspace in all cans, corrosion would be inversely proportional to the vacuum in the can. Yet there was no apparent relationship between the degree of internal corrosion and the vacuum as determined by a vacuum gauge (American Can Company). Extreme corrosion was evident in cans with a vacuum as high as 16 inches (40 cm.), others with practically no vacuum showed very little corrosion. A similar lack of relationship was found in individual lots in which all cans were put up about the same time and were stored under identical conditions. One must conclude that gaseous oxygen is not a major factor in the degree of internal corrosion of herring in plain cans. A similar conclusion was reached by Dill and Clark (1926) in a study of corrosion in canned crab, namely, that free oxygen was not a significant factor in can corrosion or in the blackening of the flesh.

DISCUSSION

As a broad generalization, Sanders (1942) states that the mechanism whereby tin protects steel against corrosion is perhaps not yet fully understood, but under conditions prevailing within non-acid products, such as fish, tin is cathodic to iron and protects it merely by covering it up. The more complete coverage, therefore, of steel by the tin, the greater the protection offered. Unfortunately, it has been found that complete coverage of the steel is never attained because of the occurrence of porosity in the coating of the tin plate. Hoare (1937) found that the number of pores per 100 sq. cm. falls from 8,000 to 300 as coating thickness is increased from 1 to 3 pounds per base box and continues to fall, but more slowly, as the thickness is still further increased. With the Japanese invasion of Malaya, the Dutch East Indies and Burma, supplies of tin to the Allied Nations from those areas were cut off. In consequence, as a conservation measure the weight of tin plate was reduced. In the U.S.A. the weight of tin plate per base box was reduced from 1.5 to 1.08 pounds (680 to 490 g.) (Lueck 1942). It is a reasonable assumption, therefore, that the number of pores in the tin plate of cans at the time this investigation was carried out was several thousand per 100 sq. cm. The reduction in the weight of tin plate, with consequent increased porosity, would be conducive to more rapid internal corrosion in plain or unenameled cans.

The mechanism of corrosion of tin plate in tin cans has been studied by many investigators. There is general agreement that corrosion takes place as a result of exposure of the steel plate to the can contents, through the pores or discontinuities in the tin coating, with consequent formation of an electrocouple.

Schmidt-Nielsen and T. Bjorgum (1941) state that corrosion in tin containers of fish takes place in two stages. In the first, differences of potential arise between the different areas of the tin surface, depending on whether they are in contact with air, the liquid or the solid contents. In the last case, the tin coating

is quickly dissolved. Oxygen functions as a depolarizer in this process and, hence, the amount of tin dissolved is proportional to the amount of oxygen present. In the second stage, when the oxygen is exhausted, the whole surface is attacked, the area of exposed iron being the cathode. Polarization retards this process and, consequently, the amount of oxygen present in the can is a limiting factor in corrosion. Hoar (1936) noted that, in the corrosion of steel by neutral media, oxygen was the controlling factor. Our findings showed no apparent relationship between available oxygen and corrosion.

It has been observed that the rate of corrosion of herring in plain cans is much more rapid than that of mackerel under similar conditions. Corrosion and iron sulphide formation was found to take place in canned herring packed from strictly fresh fish. Corrosion, therefore, is not primarily caused by chemical or bacterial decomposition of the fish before canning, although Dill and Clark (1926) have found that blackening in crustacea was hastened by bacterial decomposition. Dill and Clark (1926) noted, however, that bacterial decomposition appeared to cause no corrosion in canned Pacific salmon. They cite as examples six cans of salmon (plain) varying from normal to putrid odour in which all cans were only slightly corroded. It would seem that the rate of corrosion in canned herring is related, in some manner, to the chemical composition of the flesh. Lovern (1938), in a study of the composition of Atlantic herring fat, found an abnormally high content of C_{22} acids, unusually unsaturated C_{16} and C_{18} acids, and unusually saturated C_{20} and C_{22} acids. How this unusual composition of the fat would effect corrosion is problematical, but the possibility exists. There is some indication that corrosion from herring with low fat content is more rapid than from herring with high fat content; however, we have insufficient data to substantiate this. Portnov (1936) observed that fat in preserved food retards the solution of tin.

Examination at this laboratory of hundreds of cans of herring stored for varying periods has shown that the use of enamel-lined cans is completely effective in prevention of corrosion in canned herring. The observation made by Lipsett (1933) that in acid foods, especially fruits, tin appears to have a protective action on the exposed pin holes of iron, and that in enamel-lined cans corrosion is more serious than in plain cans, is not applicable to canned fish. (This "enamel" used in lining tin-plate food containers is really a lacquer. The term "enamel" is used in the can-making industry to differentiate the baked coating used on tin plate from the air-dried coating commonly known as lacquer (Gosner 1936)).

The theory is advanced here that a major factor contributing to corrosion of herring in plain cans is the presence of large amounts of sulphide. This sulphide arises as a result of the catalytic action of tin on the herring, and while decomposition of the flesh, previous to canning, may augment the amount of sulphide, there are adequate amounts present at all times in the can for the deposition of a large quantity of iron sulphide. This theory is supported by the findings of Dill and Clark (1926), who observed from experimental canning, that about ten times as much sulphide was present in spiny lobster (*Panulirus interruptus*) contained in plain cans as in glass jars. Yet all lobster meat had been handled in the same manner. Homans (unpub.) also found large amounts of

sulphide in mackerel in plain cans, but very little in mackerel in enameled cans packed under identical conditions.

It would seem that the degree of sulphide formation is unrelated to the initial concentration of sulphur in the flesh. McCance and Widdowson (1940) gave the sulphur content of raw herring as 191 mg. per 100 g. of fish, a value which does not exceed that of other fish, including mackerel and Pacific salmon (Lueck 1943). Yet, apart from the formation of limited amounts of tin sulphide—a condition known as spangling—corrosion is practically unknown in Pacific salmon put up in plain cans. The catalytic action of tin on the sulphur-bearing proteins with production of sulphide seems to be limited, therefore, to certain species, such as crustacea, herring, gaspereau, and, to a lesser extent, mackerel.

The concentration of tin in corroded cans will be influenced by various factors, such as the temperature at which the cans are held, the acidity of the food, the length of storage and the extent of the areas of the can interior involved. That other factors are involved in the dissolution of tin and, therefore, the degree of corrosion, is shown by Shrader (1939), who cites as evidence the amount of tin taken up by string beans as contrasted to that of lima beans. The tin content of string beans was found to be 124 parts per million after two months' storage, and 434 after two years and six months; whereas the tin content of lima beans after nine months' storage was 80 parts per million, and 173 after two years and nine months. Back (1933) states that tin is a normal constituent of animal tissue, having been found by Bertrand and Curirea in fresh tissue of beef, horse and mutton, and that traces have been found by spectrographic methods in fresh fish.

In the opinion of Cronshaw (1937), the average tin content of canned foods reaching the consumer is about 30 parts per million. This figure is probably sufficiently high for enameled cans, but the tin content of food in plain cans may be higher than this. Morris and Bryan (1931) gave the tin content in parts per million of canned salmon as 111, lobster 380, mackerel 78 and bluebacks as 111, but failed to state whether the cans were enameled or unenameled. The author analysed lobster from a C-enamel can stored for about 8 years at room temperature, and found the tin content to be 14 parts per million. The contents of two tins of mackerel, also in C-enamel cans and stored for 10 years, had a tin concentration of 25 and 40 parts per million. Other investigators have found that enameled cans effectively inhibit corrosion and the solution of tin. Such corrosion as does take place is generally confined to the cover seam. The general trend today, especially in the canning of fruits and vegetables, is toward the use of cans lined with a suitable enamel. This is especially necessary in present-day cans with the reduced weight of tin plate.

Tin poisoning is practically unknown, and feeding experiments, both on animals and man, have failed to demonstrate any harmful effects after the ingestion of far larger amounts than could possibly be taken up from tin cans. Dack (1943) cites an experiment in which four subjects ingested a total of 2274 to 2942 mg. of tin with an average daily intake ranging from 426 to 490 mg. Neither during the tin-feeding period nor during the post-tin feeding did any of the subjects show any evidence of illness or discomfort. Nevertheless, in Great

Britain a maximum tin content of 2 grains per pound (286 parts per million) has been set as the limit, and above this limit a product is considered "potentially injurious to health" (Cronshaw 1937). Glaister (1942) points out that salts of tin cause gastro-intestinal irritation such as nausea, vomiting, pain in the abdomen, diarrhoea and prostration. He states that 2 grains (130 mg.) may constitute a poisonous dose. However, it has been shown by Goss (1917) that tin combines with protein to form an irreversible, insoluble compound which is quite harmless and which is excreted.

The problem of toxicity of tin is well summarized by Morris and Bryan (1931) who suggest that "In the present state of knowledge it would not be justifiable to say that small amounts of tin in food are entirely without effect upon metabolism. In any case, it is obvious that if tin is absorbed, it will have to be dealt with in the body and excreted, and on general principles it is undesirable to impose upon the tissues of the body an extra strain which they need not be called upon to bear."

In the case of iron it has been shown that the small amounts which may be taken up by the can contents are harmless. Moreover, the formation of iron sulphide, discolouration of the can contents, and strong metallic odour, safeguard the consumer against pronounced metallic contamination by iron.

It has not been found possible to predict, with any degree of accuracy, the maximum period that canned herring, showing various degrees of internal corrosion, may be stored before it becomes inedible. As previously pointed out in this paper, many factors determine the rate of corrosion. As a generalization, it was found that if examination of representative samples from a lot revealed that the extractable iron content of the fish, and spot tests for iron on the can interior, were practically negative, the lot remained edible for several months when stored at room temperature. If, however, there was evidence that the steel plate was exposed, even in the absence of definite metallic odour and flavour from the contents, corrosion was very rapid and the lot was considered unfit after two or three months.

SUMMARY

It has been shown that corrosion in canned herring is proportional to the concentration of extractable iron. A rapid method for estimation of extractable iron is presented by which an analysis can be made in 20 to 30 minutes.

The theory is advanced that rapid corrosion in canned herring (plain cans) is due largely to the production of large amounts of sulphide caused by the catalytic action of tin on the sulphur-bearing proteins. Corrosion can be eliminated by the use of cans lined with suitable enamel.

The storage period during which the contents of partly corroded cans will remain edible cannot be predicted with any degree of accuracy.

REFERENCES

Back, S. *Food Manuf.*, **8**, 381-384, 1933.

Baker, I., M. Miller and R. S. Gibbs. *Ind. Eng. Chem., Anal. Ed.*, **16**, 269-271, 1944.

B. D. H. Book of organic reagents, 8th ed. 1-55, British Drug Houses, London, Eng., 1941.

BRENNERT, S. *Internat. Tin Res. Dev. Counc., Tech. Pub.* D, **2**, 1-27, 1935.
CRONSHAW, H. B. The wholesomeness of canned foods. *Internat. Tin. Res. Dev. Counc., Informat Circ.*, **2**, 1-19, 1937.
DACK, G. M. Food poisoning. 1-138, University of Chicago Press, Chicago, 1943.
DILL, D. B. AND P. B. CLARK. *Ind. Eng. Chem.*, **18**, 560-563, 1926.
GLAISTER, J. Medical jurisprudence and toxicology, 7th ed. 1-671, E. and S. Livingstone, Edinburgh, 1942.
GOSNER, B. W. Tinplate and tin cans in the United States, 1-144. *Internat. Tin. Res. Dev. Counc. Bull*, **4**, 1-144, 1936.
GOSS, B. C. *Ind. Eng. Chem.*, **9**, 144-148, 1917.
HOAR, T. P. *Internat. Tin. Res. Dev. Counc., Tech. Pub.* A, **30**, 1-11, 1936.
HOARE, W. E. *Internat. Tin. Res. Dev. Counc., Tech. Pub.* A, **59**, 1-22, 1937.
JACKSON, C. J., G. R. HOWAT, AND T. P. HOAR. *Internat. Tin Res. Dev. Counc., Tech. Pub.* A, **49**, 1-10, 1936.
LIPSETT, S. G. *Canad. Chem. Met.*, **17**, 171-174, 1933.
LOVERN, J. A. *Biochem. J.*, **32**, 676-680, 1938.
LUECK, R. H. Metal container changes in the interest of tin conservation, 1-23. Can Manufacturers Institute, New York, N.Y., 1942.
The canned food reference manual. 2nd ed. 1-552, American Can Company, New York, N.Y., 1943.
MCCANCE, R. A., AND E. M. WIDDOWSON. The chemical composition of foods. 1-150, Chemical Publishing Co., New York, N.Y., 1940.
MORRIS, T. N., AND J. M. BRYAN. The corrosion of tin-plate containers by food products. *Gr. Brit. Food Inv. Bd. Spec. Rep.* **40**, 1-85, 1931.
PORTNOV, YA. L. *Konservnaya Prom.* **4**, 16-21, 1936 (through *Chem. Abstr.* **31**, 2307, 1937).
SANDERS, R. K. *Food Manuf.*, **17**, 229-231, 1942.
SCHMIDT-NIELSEN, S., AND T. BJORGUM. *K. Norske Videnskab. Selskabs. Forh.* **13**, 135-138, 1941. (Through *Chem. Abstr.*, **37**, 3513, 1943.)
SHRADER, J. H. Food control. 1-513, John Wiley and Sons, New York, N.Y., 1939.
SWANK, H. W., AND M. G. MELLON. *Ind. Eng. Chem., Anal. Ed.*, **10**, 7-9, 1938.
TOMPSETT, S. L. *Biochem. J.*, **28**, 1536-1543, 1934.

Microorganisms From Atlantic Cod

By Frances E. Dyer

Atlantic Fisheries Experimental Station
Halifax, N.S.

(*Received for publication February 18, 1947*)

ABSTRACT

Five hundred and ninety-five organisms isolated from six cod were members of the genera *Micrococcus, Microbacterium, Achromobacter, Pseudomonas, Flavobacterium, Proteus, Alkaligenes,* moulds and yeast-like organisms. A new species, *Microbacterium piscarium,* is described. 98% of the cultures produced acid from carbohydrates, 60% lysed fish muscle, 29% reduced trimethylamine oxide to trimethylamine, and 45% were chromogenic.

Spoilage of fish is caused largely by bacteria. For this reason, the kinds of bacteria on the fish and their characteristics are important in improving methods of handling and preservation of fish products.

Among the workers contributing to our knowledge of the bacteriology of sea fish have been Spence (1927) studying haddock, Harrison (1929) with halibut, Reed and Spence (1929) and Stewart (1932) with haddock, Bedford (1933) with halibut, Thjotta and Somme (1938) with cod, Snow and Beard (1939) with salmon, Wood (1940) with salmon, flathead, mullet and whiting, Aschehoug and Vesterhus (1943) with herring, and Kiser and Beckwith (1944) with mackerel. These ten studies in different parts of the world all showed *Achromobacter, Flavobacterium* and *Micrococcus* common on sea fish. In most cases *Pseudomonas* was also considered important. *Bacillus, Sarcina, Staphylococcus, Lactobacillus, Streptococcus, Kurthia, Proteus, Rhodococcus, Serratia* and members of the colon-aerogenes group were each found in several of the investigations. We thus have a general picture of the flora of sea fish with *Micrococcus, Achromobacter, Flavobacterium,* and *Pseudomonas* predominating and other genera also present.

The bacteria are present on the skin, gills, and, especially in feeding fish, in the digestive system. Although Gee (1927) reported the isolation of *Bacillus mesentericus vulgatus* from the muscle of fresh haddock most workers have found fish muscle sterile (Browne 1918, Obst 1919, Hunter 1920a, Proctor and Nickerson 1935, Shewan 1942, Dyer, Dyer and Snow 1946). In properly handled fish the muscle stays sterile until the fish are badly spoiled and spoilage takes place at the surfaces, nutrients for the bacteria diffusing out to the surfaces and products of bacterial metabolism diffusing back into the muscle to cause off-flavours and odours (Dyer, Dyer and Snow 1946).

The bacteria present on the fish are mostly aerobes. Hunter (1920b), Snow and Beard (1939) and Kiser and Beckwith (1944) were unable to find any anaer-

J. Fish. Res. Bd. Can., 7 (3) 1947.
Printed in Canada.

obes in the intestines of sea fish. Reed and Spence (1929) and Shewan (1938, 1942) found *Clostridium* always present in the intestines but never in the slime from the skin.

Wood (1940) found a generic succession during fish spoilage; the *Micrococcus* and *Flavobacterium* which predominated when the fish were overgrown by *Achromobacter* and *Pseudomonas* by the time the fish reached the retail dealers.

In the present study 595 cultures isolated from live cod caught by handline in Terrence bay, N.S., and from cod caught on trawl lines and landed by the fishing vessels at Halifax have been identified.

COLLECTION OF SAMPLES

Sterile test tubes were used to scrape 2 to 5 cc. of slime from live fish as they were lifted from the sea or from fish after they were landed at the plants. Feces were collected by pressing the belly of the live fish until 1 to 5 cc. were expressed into the test tubes.

ISOLATION OF CULTURES

Dilutions were made in sterile tap water and plated in a nutrient agar (Dyer, Dyer and Snow 1946) containing Bacto-peptone, Bacto-beef extract, Bacto-yeast extract, each 0.2%, Bacto-glucose 0.1%, NaCl c.p. 0.5%, K_2HPO_4 0.1%, Bacto-agar 1.5%, pH adjusted to 7.2 before autoclaving.

Plates with approximately 100 colonies were used for isolations. All the colonies from a single plate were transferred to slants of the same medium. Five series of cultures were isolated from slime and one from feces:

130	cultures	from	slime	of	vessel	fish,	incubated 3 days at 37°C.
110	"	"	"	"	"	"	1 week at 20°C.
60	"	"	"	"	"	"	5 weeks at 20°C.
75	"	"	"	"	"	"	3 weeks at 4°C.
112	"	"	"	live	"	"	1 week at 20°C.
108	"	"	feces	live	"	"	1 week at 20°C.

IDENTIFICATION OF CULTURES

The rod-shaped bacteria isolated were classified according to Bergey, Breed, Murray and Hitchens 1939, the cocci according to the 6th edition of the same publication which is now in press. The biochemical reactions of the cultures were determined at 20°C.

Nitrate reduction: Dyer's reagents (Dyer 1946) were used to test for nitrite after abundant growth was obtained in Zobell's medium (Zobell 1932). Zinc dust was added to the negative tubes to detect cultures which had used up all the nitrate and nitrite produced from it as suggested by Zobell (*ibid.*).

Gelatin decomposition: Giant colonies were grown two to four days on Frazier's gelatin agar plates (Frazier 1926) and flooded with 1% tannic acid to precipitate gelatin decomposition products. 0.01% Bacto-beef extract was substituted for beef infusion in the original medium. Doubtful cultures were checked by 100 days' incubation in 4% and 10% Bacto-gelatin.

Ammonium phosphate utilization: The use of $NH_4H_2PO_4$ as sole nitrogen source was determined in Breed's medium (Breed 1928) with four weeks' incubation.

Chromogenesis: Pigment was noted on the stock agar at 18 days and 5 weeks.

Presence of unrease: Cultures with Proteus-like characteristics were tested for urea decomposition by Anderson's rapid method (Anderson 1945). Anderson's medium without urea and adjusted to the same pH with the glass electrode was used as a control and a stock culture of *Proteus vulgaris* included in each run. Thirty days' incubation in Breed's urea medium (Breed 1928) was used to separate *Micrococcus ureae* and *M. freudenreichii, Alkaligenes metalkaligenes* and *A. ammoniagenes*.

Action in litmus milk: The cultures were grown 30 days in Difco-litmus milk (later Difco-Purple milk) to determine their effect on this medium.

Indol production: Kovács reagent (Kovács 1928) and Erhlich's reagent (Salle 1939) were used to test for indol production in 1% Bacto-tryptone broth after abundant growth was obtained.

Fermentation of sugars: Agar slants of the following composition were used: Bacto-peptone, Bacto-yeast extract and K_2HPO_4 each 0.1%, NaCl c.p. 0.5%, Bacto-agar 1.5%, carbohydrates being tested 1%, phenol red or Andrade's indicator to give a distinct but pale colour. Reaction was adjusted to pH 7.2 and the medium was autoclaved 30 minutes at 10 lb. pressure. The tubes were inoculated by streaking the slant and stabbing into the butt. They were examined every two days for two weeks, then once a week for two weeks longer.

Motility: Growth from 16- to 24-hour slants was examined by the hanging drop technique with a magnification of 900*x*.

Trimethylamine oxide reduction: Production of trimethylamine from trimethylamine oxide was determined by the method of Wood and Baird (1943).

Morphology and gram reaction: Burke's modification (Kolmer and Boerner 1941) of Gram's stain was used to determine the gram reaction and morphology. Aqueous safranin and aqueous crystal violet were also used in studying the morphology of the cultures.

The number of bacteria isolated from sea fish probably depends on the medium used, the plating temperature and the temperature of incubation. Counts on the stock agar used in the present study were made after three days at 20°C.

Counts on the slime of twelve fish as they came from the sea varied from 44,000 to 350,000 per g. slime, with an average of 160,000 per g. Counts on the feces of eight fish varied from 0 in the non-feeding season to 6,000 to 26,000 per g. feces in mid-July with an average of 24,000. Counts on nine fish from the vessels ranged from 80,000 to 800,000 per g. slime with an average of 403,000 per g.

RESULTS

Cocci predominated in the flora of both the slime and feces of the cod studied. The 487 cultures from the slime were classified morphologically as 74% cocci, 20% bacilli and 6% yeast-like organisms. The 108 cultures from cod feces consisted of 66% cocci, 20% bacilli, 9% yeast-like and 5% mold-like organisms.

All the cocci isolated were members of the genus *Micrococcus*. Table I shows the *Micrococcus* species isolated and their number and source. *M. epidermidis*

predominated in all the isolations except those from feces, followed by *M. varians*, *M. candidus*, and *M. aurantiacus*. *M. epidermidis* colonies were more than three times as numerous as those of any other species.

TABLE I. *Micrococcus* species isolated from cod slime and feces

Species	Number of organisms isolated from						Total
	Vessel fish				Live fish		
	Slime	Slime	Slime	Slime	Slime	Feces	
Incub. temp.	37°	20°	20°	4°	20°	20°	
Incub. time	3 days	1 week	5 weeks	3 weeks	1 week	1 week	
M. epidermidis	58	28	15	29	22	11	163
M. varians		16	8	24	2	3	53
M. aurantiacus	2		8	1	18	21	50
M. candidus	7	12	6	2	11	12	50
M. freudenreichii	4	9	1	3	2	3	22
M. pyogenes var. *albus*	10	1	2	1	2	5	21
M. pyogenes var. *aureus*	1		2		8	8	19
M. rubens	4	1			5	1	11
M. caseolyticus	4	1	1	2	1		9
M. cinnebareus					5	4	9
M. citreus		3	1		3		7
M. rhodochrous					6		6
M. conglomeratus	1	1		1	1		4
M. ureae					2	2	4
M. roseus			1				1

The rod-shaped bacteria isolated were members of the genera *Proteus*, *Achromobacter*, *Flavobacterium*, *Alkaligenes*, *Pseudomonas* and *Microbacterium*. Table II lists the species found and the number and source of each species. *Microbacterium*, *Achromobacter* and *Pseudomonas* occurred more often than *Flavobacterium*, *Proteus* and *Alkaligenes*. *Achromobacter candicans* was the most common rod-shaped bacterium. Twenty-eight strains of a new rod-shaped species belonging to the genus *Microbacterium* have been studied. The new species is described below. Four strains of an unknown orange-coloured, trimethylamine-oxide-reducing *Microbacterium* and one strain of *M. lacticum* were also isolated.

This is the first recorded isolation of *Microbacterium* from sea fish. All the strains were small rods, non-motile, without endospores, granular and gram-positive. All produced acid from carbohydrates. Surface growth at 20°C. was very abundant but growth in stabs below the surface very scanty. Catalase was present, ammonia was produced from peptone and litmus milk was made alkaline by the *Microbacterium* cultures.

Of the 38 yeast-like organisms 13 were from live fish slime, 10 from feces, 10 from vessel fish after incubation at 4°C. and 2 from vessel fish at 20°C. None was isolated at 37°C. The yeast-like organisms were not classified but 31 were

probably rose-coloured Torula and 6 white Torula. One was a dark brown yeast producing four spores. The six moulds seemed to be of only one kind. They were very numerous on some plates from feces which were not used for isolations but were not present on the plates from slime at any of the incubation temperatures.

There was little difference in the isolations made at 37° and 20°C. from fish landed by the vessels. Of the cultures studied, 30% were rods and 70% and 68.8% respectively were cocci with 1.2% at 20°C. yeast-like and mould-like organisms. At 4°C., closer to the temperature at which fish is usually stored, 84% of the isolations were cocci, only 2.6% rods and 13.3% yeast-like and mould-like organisms.

TABLE II. Rods isolated from cod

Species	Number of organisms isolated from						Total
	Vessel fish				Live fish		
	Slime	Slime	Slime	Slime	Slime	Feces	
Incub. temp.	37°	20°	20°	4°	20°	20°	
Incub. time	3 days	1 week	5 weeks	3 weeks	1 week	1 week	
Microbacterium piscarium	12	15	1				28
M. lacticum		1					1
M. sp.			4				4
Achromobacter candicans	7	6			2	2	17
A. guttatum			1				1
A. liquidium					2	1	3
A. delicatulum	2						2
A. healii	4						4
A. dendriticum	2					1	3
Pseudomonas sp.	5	6	1		5	6	23
Flavobacterium maris	1		2	1	2	3	9
F. solare	1	1	1			3	6
F. proteus			2				2
F. lutescens				1			1
F. drobachende		1					1
Proteus vulgaris	2	3	1			2	8
P. morganii		2					2
Alkaligenes ammoniagenes	2		1			2	5
A. metalkaligenes	1	2				2	5

DESCRIPTION OF *MICROBACTERIUM PISCARIUM*, NOV. SP.

Non-motile rods 1.5 to 2.5 × 0.6μ. Highly granular and therefore apt to be confused with micrococci. Granules only retain gram stain.

Agar streak—abundant, flesh coloured, glistening, villous.

Agar colonies—flesh-coloured, umbonate, glistening, opaque, margin lobate.

Agar stab—slight growth.

Litmus milk—alkaline, may be peptonized or reduced.

Gelatin usually not decomposed.
Nitrate may or may not be reduced.
Indol not produced.
Catalase present.
Produces acid from fructose, inositol, usually from dulcitol, glycerol, raffinose and inulin. May or may not produce acid from maltose and mannitol. No acid from lactose or d-mannose. Acid production usually rapid, less than twenty-four hours.
Ammonia formed from peptone.
Ammonium phosphate may or may not be used as sole nitrogen source.
Trimethylamine oxide not reduced to trimethylamine.
No fluorescence.
Source—isolated from fish slime.
The variation among the different strains isolated is shown in Table III.

TABLE III. Biochemical reaction of 28 strains of *Microbacterium piscarium*

	Per cent positive	Per cent negative
Litmus milk alkaline	100	0
Gelatin decomposed	3	97
Nitrate reduced	45	55
Indol from tryptone	0	100
Catalase present	100	0
Fructose fermented	100	0
Inositol "	100	0
Raffinose "	83	17
Glycerol "	85	15
Dulcitol "	91	9
Inulin "	75	25
Maltose "	66	34
Mannitol "	44	56
Lactose "	0	100
Mannose "	9	91
Ammonia from peptone	100	0
$NH_4H_2PO_4$ as N source	60	40
Trimethylamine oxide reduced	0	100
Fluorescence	0	100

RELATION TO SPOILAGE

After the death of a fish the carbohydrate (glycogen) of the muscle is converted to lactic acid by autolytic enzymes. Microorganisms then bring about spoilage changes by using some of the tissue constituents and by producing waste products which spoil the flavour and odour of the flesh. Various reactions are involved in the normal spoilage of cod muscle. The lactic acid is broken down and trimethylamine oxide is reduced to trimethylamine. Later proteolysis takes

place and compounds with foul odours are produced. By this time, the flesh is unfit for consumption. Sometimes the appearance of the fish is spoiled by chromogenic bacteria, usually with a yellow or yellow-green pigment.

To get an indication of the spoilage potential of the microorganisms cultures were tested for trimethylamine oxide reduction to trimethylamine, lysis of fish muscle, production of indol and chromogenesis. The medium used for lysis of fish protein was similar to the media of Sanborn (1930) and Wood (1940). It consisted of a suspension of fish muscle in 1.5% Bacto-agar. The muscle suspension was prepared by comminuting muscle which had been sterilized (by exposure to 25 cm. partial pressure of ethylene oxide for 15 minutes after evacuation to 70 cm. mercury) in sterile water in the Waring Blendor. One ml. of the suspension was added to each petri plate of agar and mixed before the agar solidified. Positive cultures produced a clear zone around the streak or colony. More than half the cultures (table IV) lysed fish protein and nearly a third reduced trimethylamine oxide to trimethylamine. Nearly half produced yellow, orange or red pigments but very few formed indol. Most of the yeasts and moulds were chromogenic and proteolytic but did not reduce trimethylamine oxide. Approximately 58% of the 551 bacterial strains were proteolytic, 41% chromogenic and 31% reduced trimethylamine oxide.

TABLE IV. Spoilage potential of cod flora

	No. cultures	Per cent chromo-genic	Per cent trimethyl-amine +	Per cent proteo-lytic	Per cent indol +	Per cent carbohy-drate +
Slime from live fish...	107	53	14	26	..	99
Feces from live fish...	96	49	16	25	..	99
Slime from fish from vessels.....	375	30	36	54	..	97
Micrococcus.....	453	40	31	58	1	100
Rods.....	125	41	32	58	0	95
Total number cultures.	622	45	29	60	0.8	98

The sugar fermentations used in the identification of the cultures showed that approximately 98% produced acid from various carbohydrates, possibly an indication of their ability to attack the carbohydrate in fish muscle. More of the bacteria from the live fish were chromogenic but the flora of the fish from the vessels were more active in reducing trimethylamine oxide and lysing fish muscle. This is probably because the bacteria able to use fish muscle protein and to reduce trimethylamine oxide would tend to increase more rapidly than the bacteria unable to use these materials for their growth and metabolism.

COMPARISON WITH SIMILAR INVESTIGATIONS

The results have been compared with those of other authors (table V). The chief points of interest are the predominance of the genus *Micrococcus* and the

presence of *Microbacterium* on the cod studied in this investigation. *Achromobacter*, *Flavobacterium* and *Pseudomonas* were present in smaller numbers than in other investigations. In agreement with Wood (1940) few indol formers were found. Of the *Micrococcus* cultures from fish, 31% reduced trimethylamine oxide to trimethylamine. Baird and Wood (1944) found that none of the 97 identified *Micrococcus* cultures isolated from land sources which they studied reduced the oxide. Unidentified *Micrococcus* cultures from fish which reduced trimethylamine oxide have been reported by Tarr (1939) and Sigurdsson and Wood (1942).

TABLE V. The flora of sea fish according to various authors, as shown by per cent of isolations in the various generic groups

Author	*Micrococcus*	*Achromobacter*	*Flavobacterium*	*Pseudomonas*	*Bacillus*	Miscellaneous
Reed and Spence (1929)...	4	23	8	22	24	18
Stewart (1932).....	22	57	11	5	..	5
Bedford (1933).....	16	34	30	..	..	20
Shewan (1938).....	24	43	13	11	..	9
Thjotta and Somme (1938)	14	48	25	5	..	8
Wood (1940).....	48	19	17	7	9	..
Aschehoug and Vesterhus (1943).....	16.7	24.5	17.7	40	..	1.1
Snow and Beard (1939)...	12.6	53.7	4.9	8.4	1.5	18.8
Dyer.....	72.8	4.8	3	3.7	..	15.7

SUMMARY

From cod, 595 cultures were isolated. Of these, 163 were strains of *Micrococcus epidermidis*, 266 were strains of 15 other species of *Micrococcus*, 125 were rod-shaped bacteria, members of the genera *Microbacterium*, *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Proteus* and *Alkaligenes*, 38 were yeast-like and 6 mould-like organisms.

A new species of *Microbacterium* (28 strains) was isolated from cod slime.

Of the cultures from slime and feces, 98% fermented carbohydrates, 60% lysed fish muscle, 29% reduced trimethylamine oxide to trimethylamine and 45% were chromogenic.

ACKNOWLEDGEMENT

The author wishes to thank Dr. R. S. Breed for the galley proof of the section on the cocci of Bergey's Manual, 6th edition, which has been invaluable in the present investigation.

REFERENCES

ANDERSON, T. G. *Science*, **101**, 470, 1945.

ASCHEHOUG, V., AND R. VESTERHUS. *Zbt. Bakt.* II Abt. **106**, 5-27, 1943. (Cited in *Chem. Abst.* **38**, 4006, 1944.)

BAIRD, E. A., AND A. J. WOOD. *J. Fish. Res. Bd. Can.*, **6** (3), 243-244, 1944.

BEDFORD, R. H. *Proc. Fifth Pac. Sci. Congr.*, **6**, 3715-3724, 1933.
BERGEY, D. H., R. S. BREED, E. G. D. MURRAY AND A. P. HITCHENS. Bergey's manual of determinative bacteriology, 5th ed., 1-1032, Williams and Wilkins Co., Baltimore, 1939.
BREED, A. *N.Y. Agric. Exp. Sta. Tech. Bull.*, **132**, 1-28, 1928.
BROWNE, W. W. *J. Bact.*, **2**, 417-422, 1918.
DYER, W. J. *J. Fish. Res. Bd. Can.*, **6**, 414-418, 1946.
DYER, W. J., F. E. DYER AND M. SNOW. *J. Fish. Res. Bd. Can.*, **6**, 403-413, 1946.
FRAZIER, W. C. *J. Bact.*, **11**, 80 (Abst.) 1926.
GEE, A. H. *Contr. Canad. Biol. Fish.* (N.S.) **3**, 349-363, 1927.
HARRISON, F. C. *Canad. J. Res.*, **1**, 214-239, 1929.
HUNTER, A. C. *J. Bact.*, **5**, 353-361, 1920a.
J. Bact., **5**, 543-552, 1920b.
KISER, J. S., AND T. D. BECKWITH. *Food Res.*, **9**, 250-256, 1944.
KOLMER, J. A. AND F. BOERNER. Approved laboratory technic, 1-921, 3rd ed., D. Appleton, Century Co. Inc., New York, 1941.
KOVÁCS, N. *Z. Immunitäts.*, **55**, 311-315, 1928.
PROCTER, B. E., AND J. T. R. NICKERSON. *J. Bact.*, **29**, 71 (abst.) 1935.
OBST, M. M. *J. Inf. Dis.*, **24**, 158-169, 1919.
REED, G. B. AND C. M. SPENCE. *Contr. Canad. Biol. Fish.*, **4**, 259-264, 1929.
SALLE, A. J. Fundamental principles of bacteriology, 1-679, McGraw Hill, New York, 1939.
SANBORN, J. R. *J. Bact.*, **19**, 375-382, 1930.
SHEWAN, J. M. *J. Bact.*, **35**, 397-407, 1938.
Chem. and Ind., **61**, 312-314, 1942.
SIGURDSSON, G. J., AND A. J. WOOD. *J. Fish. Res. Bd. Can.*, **6** (1), 45-52, 1942.
SNOW, J. E. AND P. J. BEARD. *Food Res.*, **4**, 563-585, 1939.
STEWART, M. M. *J. Mar. Biol. Assn.*, **18**, 35-50, 1932.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1939.
THJOTTA, T., AND O. M. SOMME. *Acta. Path. Microbiol. Scand. Suppl.* **37**, 514-526, 1938. (Cited in *Chem. Abst.* **33**, 678, 1939).
WOOD, A. J., AND E. A. BAIRD. *J. Fish. Res. Bd. Can.*, **6**, 194-201, 1943.
WOOD, E. J. F. *Coun. Sci. Ind. Res. Australia Pamph.* **100**, 1-92, 1940.
ZOBELL, C. E. *J. Bact.*, **24**, 273-281, 1932.

Control of Rancidity in Fish Flesh

I. Chemical Antioxidants

By H. L. A. Tarr
Pacific Fisheries Experimental Station
Vancouver, B.C.

(*Received for publication August 10, 1946*)

ABSTRACT

The development of rancidity in the naturally occurring fats of frozen fish was retarded by treatment with ascorbic acid (0.05%), ethyl, n-propyl, n-butyl or hexyl gallates (0.01 to 0.05%), and cysteine hydrochloride (0.05%). Ethanol ammonium gallate (0.02%), dodecyl thiodipropionate (0.05%), thiourea (0.05%), citric and tartaric acids (0.02%) were ineffective. The loss of surface red colour which occurred during storage of coho and red spring salmon was largely prevented by pre-treatment with 0.02% of ethyl or propyl gallate. Both NaCl and $NaNO_2$ acted as pro-oxidants in frozen fish. In unfrozen salmon flesh stored at 0° C. both ethyl gallate and $NaNO_2$ retarded fat oxidation and bacterial increase.

During recent years there has been a growing literature concerning the use of chemical antioxidants for retarding the development of rancidity in isolated animal and vegetable fats and oils, but less attention appears to have been paid to the possible direct application of such compounds to animal flesh as a means of controlling oxidation of the indigenous fats.

Bahr and Willie (1933) suggested that certain phenolic compounds such as hydroquinone, eugenol or α-naphthol be added to the salt or brine used in fish curing in order to retard "rusting." Oat flour has been found to inhibit the onset of rancidity in salted mackerel (Peters and Musher 1937; Lemon, Stansby and Swift 1937 a and b). However, Australian work (Anon. 1940, pp. 57-58) suggests that the antioxidant effect of oat flour when used on fish fillets is very weak, and is confined more to its action in slightly depressing the peroxide value of the fat than to causing an actual improvement in flavour of the stored fish. Since the publication of short preliminary articles dealing with the present work (Tarr 1944 a and b, 1945), Silver (1945) has shown that development of oxidative rancidity in brine-cured mackerel and herring can be retarded considerably if the cured fish is dipped in oil containing 0.2 per cent nordihydroguaiaretic acid (NDGA) prior to storage. Recently Smith, Brady and Comstock (1945) have shown that NDGA, gossypol and a mixture of d-isoascorbyl palmitate, soybean lecithin and tocopherols can be employed to retard the onset of rancidity in sliced bacon.

J. Fish. Res. Bd. Can., 7 (3) 1947
Printed in Canada

MATERIALS AND METHODS

Antioxidants

Ethyl gallate. This compound was prepared by esterifying technical gallic acid (Beilstein 1927, p. 484). After two successive crystallizations from water and drying at 105° C. pale brown crystals of the anhydrous compound (M.P. 153-155°) were obtained. In one experiment the "Progallin A" of Nipa Laboratories was used.

n-Propyl gallate. Heyden Chemical Corporation. In one experiment "Progallin P" of Nipa Laboratories was used.

n-Butyl gallate. From Dr. C. H. Lea, Cambridge, England.

Hexyl gallate. Heyden Chemical Corporation.

Ethanol ammonium gallate. Silmo Chemical Corporation.

l-Ascorbic acid. Merck and Co., U.S.P. grade.

Cysteine hydrochloride. Eastman Kodak Co.

Thiourea. Eastman Kodak Co.

Dodecyl thiodipropionate. Du Pont de Nemours.

Sodium gallate. Aqueous solutions of Merck's U.S.P. gallic acid were adjusted to pH 6.0 with 1.0 N NaOH.

Preparation of Samples

Only strictly fresh fish were used and the antioxidants were incorporated as follows.

MINCED FLESH

Salmon (*Oncorhynchus* species) and black cod (*Anoplopoma fimbria*) were filleted and the fillets were skinned, while herring (*Clupea pallasii*) were scaled and gibbed. The flesh was comminuted with a Universal mincer having a plate with 4.5-mm. diameter holes. Antioxidants, NaCl or $NaNO_2$ were incorporated into the minced flesh by adding a 1% aqueous solution of the chemical slowly and beating the flesh in a bowl for from 2 to 3 minutes with Mixmaster beaters at speed 3. The 1% solutions of dodecyl thiodipropionate, hexyl gallate, n-butyl gallate, and n-propyl gallate were added while warm because these compounds are either sparingly soluble or almost insoluble in water at ordinary temperatures. In early experiments in which steam-extracted fat was used for determination of peroxide values, 200-g. samples of the minced flesh were wrapped in M.S.T. (moisture proof, heat sealing, transparent) cellophane and stored. However, for most of the experiments 25-g. samples were placed in 6-oz. (170-g.) wide mouth, screw-capped glass jars and then frozen and stored.

FILLETS AND SPLIT FISH

The fish was immersed for 1 minute at about 20° C. in a watery solution of the antioxidant or salt used, and drained for 5 minutes on a wire mesh screen prior to wrapping in M.S.T. cellophane, freezing and storing. In all cases a volume of solution sufficient to ensure that there would be little change in its composition during the treatment was employed.

FREEZING AND STORAGE

All samples were frozen in still air at the temperature at which they were to be stored. They were stored either in cabinets at −5, −10 or −20° ± 1° C., or in a large room the temperature of which varied from −24 to −32° C. with a rough average of −28° C.

DETECTION OF RANCIDITY

Lea (1938) has pointed out that no single available chemical test is likely to prove an absolute criterion of the degree of rancidity of a fat, and this is not surprising in view of the complex chemical changes which are involved during oxidation of unsaturated fats (Black 1945). However a quantitative determination of the amount of fat peroxides present in foods such as fresh mackerel (Stansby and Lemon 1941), frozen salmon fillets (Stansby and Harrison 1942), frozen herring (Banks 1937, 1938 and 1939), edible fats (Lea 1944b) and bacon (Smith *et al.* 1945) has proved a useful comparative index of the amount of fat oxidation which has occurred during storage, and in certain cases data thus obtained have closely paralleled those obtained by organoleptic tests. For this reason a measurement of the amount of fat peroxide present in the fish flesh has been used in these experiments as a comparative criterion of the degree of rancidity. In the case of both coho (*Oncorhynchus kisutch*) and red spring (*Oncorhynchus tshawytscha*) salmon the information derived from peroxide tests has been supplemented by that obtained from quantitative measurements of the disappearance of the surface red colour of samples during storage (*vide infra*).

In early experiments (see tables 1-6) peroxide values were determined on steam extracted fat as follows. Two-hundred-gram samples of minced flesh (defrosted where frozen) were placed in 1-l. conical flasks with 200 ml. of water, and a rapid stream of steam was run into the mixture for from 15 to 30 minutes until sufficient fat was obtained. The oily layer was decanted and roughly separated from the watery phase using a small separating funnel. The oil was placed in a small centrifuge tube, heated in boiling water, and centrifuged while hot. Peroxide values were obtained using 1-g. samples of the oil in the simplified method described by Lea (1938). This method was not found to be very satisfactory, partly because rather large samples of fish were required, but mainly because it could only be used with fish flesh which was quite rich in fat. For this reason the following method, which was similar in some respects to that described by Stansby and Lemon (1941) for determining peroxide values and free fatty acids in mackerel, was used in most of the experiments.

Twenty-five g. of minced flesh in a 6-oz. glass jar were mixed thoroughly with a like quantity of pure anhydrous sodium sulphate using a strong glass stirring rod with mushroomed end, and 50 ml. of pure chloroform were then incorporated. The jar was covered with a screw cap and kept in the dark for 15 to 30 minutes. The contents were mixed well, and filtered with suction after pouring on to a filter paper which had been moistened with chloroform and sucked on to the surface of a small Büchner funnel. Twenty ml. of the clear filtrate were placed in a 200-ml. conical flask, 30 ml. of pure glacial acetic acid

and 2 drops of saturated KI solution were added, and the flask was kept in a dark place for 10 minutes. Fifty ml. of water were added and the liberated iodine was titrated with 0.002 N $Na_2S_2O_3$ using approximately 2 ml. of acid starch indicator (Platner 1944). It was found that though the end point was not reached for several minutes (probably due to slow diffusion of the iodine into the watery layer), it was quite sharp and duplicates agreed closely. The amount of fat used for each determination was calculated from the weight of oil obtained after evaporating the chloroform from a 10-ml. sample of the above filtrate. The results are expressed as ml. of 0.002 N $Na_2S_2O_3$ per gram of fat. It was found that when ascorbic acid or ethyl gallate was added to samples of minced fish flesh in from 0.02 to 0.1% concentration, the peroxide values of the fats of such samples were not affected appreciably.

Since this work was commenced publications have appeared which show that peroxide determinations which are carried out in the presence of atmospheric oxygen yield rather higher results than do similar determinations when performed in presence of an inert gas (Lea 1945; Stuffins and Weatherall 1945). However, whether this would affect *comparative* results is apparently not known.

The initial peroxide values of the fat of fresh fish flesh were invariably zero.

Determination of Gallates

Fifteen grams of minced flesh containing ethyl or n-propyl gallate were mixed thoroughly with 45 g. of anhydrous Na_2SO_4, and 40 g. of the mixture were extracted for 1 hour with 100 ml. of ether in a Soxhlet apparatus. The ether was removed under reduced pressure, the residue extracted with 65 ml. of boiling water, and the amount of gallate present in the extract determined by the colorimetric method of Mattil and Filer (1944). The extract was suitably diluted where the gallate concentration in the flesh was over 0.01%, and as the solutions were slightly cloudy a colorimeter control sample was prepared from gallate-free fish flesh. It must be noted that the ethyl and n-propyl esters of gallic acid give in this method slightly more intense colours per mole of gallic acid than does gallic acid alone, and this must be taken into account when making determinations. Filer (private communication) has observed that with n-propyl gallate the development of colour is more rapid, the colour more intense, and the absorption curve of the coloured complex is different from that of pure gallic acid.

The following recoveries were obtained in an experiment in which given percentages of ethyl gallate were incorporated in white spring salmon flesh:—

Ethyl gallate added (%) ..	0.02	0.01	0.005	0.003	0.002	0.001
% recovery.....	100	94	104	113	90	90

This indicates that a recovery of about 100 ± 6% might be expected in flesh containing 0.01 to 0.02% ethyl gallate, but that in concentrations below 0.01% the method is less accurate.

The amount of ethyl gallate in spring salmon fillets approximately 12.5 × 7.5 × 2 cm. in size (240 to 270 g.) was determined after immersing them for different lengths of time at 20° C. in 0.5% ethyl gallate solution alone and in a

20% NaCl brine containing 0.5% ethyl gallate. The following results were obtained.

Immersing period (minutes)	0.5	1	5
% ethyl gallate in fillets immersed in:			
0.5% ethyl gallate	0.016	0.024	0.040
0.5% ethyl gallate + 20% NaCl	0.021	0.027	0.080

EXPERIMENTAL

Delaying Onset of Rancidity

UNFROZEN FISH

The effect of ethyl gallate and sodium nitrite on the keeping quality of minced white spring salmon flesh stored at 0° C. was investigated. Two 200-g. samples of the flesh were used for each treatment, peroxide values being made using steam extracted fat, and direct bacterial counts by a method previously described (Tarr 1943). The various treatments employed and the results obtained are given in table I. It will be seen that 0.02% ethyl gallate prevented

Table I. Effect of ethyl gallate and sodium nitrite on bacterial increase and development of rancidity in unfrozen minced white spring salmon flesh stored at 0° C. Bacterial count is recorded as millions of bacteria per gram and P.V. = peroxide value determined on steam extracted fat.

Treatment	Bacterial count after days		P.V. after days	
	6	14	6	14
Untreated	95	670	1.1	1.0
0.02% ethyl gallate	1.8	280	0	0
0.02% $NaNO_2$	0.17	80	0.2	0.4
0.02% $NaNO_2$ + 0.02% ethyl gallate	0.05	4.8	0	0

the development of fat peroxides and also considerably retarded bacterial increase. Sodium nitrite (0.02%), the bacteriostatic action of which has been described in previous papers (Tarr 1941, 1942) inhibited bacterial growth markedly, and also prevented development of fat peroxides. Incorporation of both ethyl gallate and sodium nitrite caused greater inhibition of both bacterial growth and fat oxidation than did either of these compounds when used alone. It seems probable that the effect of nitrite in inhibiting formation of fat peroxides in unfrozen fish flesh, which is shown both in this and the following experiment, is linked up with its bacteriostatic action. Bacteria frequently accelerate fat hydrolysis and oxidation in meats (Jensen 1945). Sodium nitrite alone has been found to accelerate fat oxidation in frozen fish (*vide infra*).

Seventeen fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from a 20-lb. (9.1-kg.) white spring salmon. One fillet was examined immediately,

the remainder being treated as recorded in table II (two fillets to each treatment) and then stored at 0° F. The results show that all treatments, listed as follows in order of preference, inhibited bacterial growth: $NaNO_2$; ethyl gallate, sodium gallate; ethyl gallate plus NaCl; sodium gallate; sodium chloride. Formation

TABLE II. Effect of ethyl gallate, sodium gallate, $NaNO_2$ and NaCl on bacterial increase and development of rancidity (peroxide value, P.V.) in unfrozen white spring salmon fillets stored at 0° C. P.V. determined on steam extracted fat.

Treatment	Bacterial count after days		P.V. after days	
	7	15	7	15
Untreated	125	380	0.4	0.95
Dipped in 0.5% ethyl gallate	1.7	410	0	0
" " 0.5% sodium gallate	3.8	980	0	0
" " 0.5% $NaNO_2$	0.34	2	0	0.15
" " 20% NaCl	6.2	540	1.3	0.95
" " 20% NaCl + 0.5% ethyl gallate	0.11	210	0	0
" " 20% NaCl + 0.5% sodium gallate	1.7	640	0	0.1
" " 20% NaCl + 0.5% $NaNO_2$	0.13	2.3	0	1.15

of fat peroxides during the short storage period was entirely prevented by ethyl gallate, sodium gallate and ethyl gallate plus sodium chloride, and was retarded by $NaNO_2$, $NaNO_2$ plus NaCl and sodium gallate plus NaCl. Fat oxidation was accelerated by treating fillets with NaCl alone.

In connection with the results recorded in the above experiments it is of interest that bacterial growth has also been found to be markedly retarded by treatment of fish flesh with n-propyl gallate.

FROZEN FISH

Herring. Herring which had been stored frozen in glazed blocks for several months were defrosted, and 24 split fish were subjected to each of the treatments outlined in table III. The minced flesh from 12 fish was used for each peroxide value test. The results of this experiment (table III) show that ethyl gallate proved a good antioxidant, especially in the higher concentrations employed,

TABLE III. Effect of ethyl gallate and sodium gallate on development of rancidity in frozen split herring stored at −5° C. Peroxide value (P.V.) determined on steam extracted fat and initially = 2.0.

Treatment	P.V. after days	
	46	117
Untreated	17.6	35.7
Dipped in 1% sodium gallate	13.0	25.6
" " 0.5% sodium gallate	17.6	25.6
" " 0.1% " "	17.0	26.4
" " 1.0% ethyl gallate	2.2	3.1
" " 0.5% " "	2.0	5.7
" " 0.1% " "	6.5	10.4

but that sodium gallate was practically inactive in this respect. Further experiments were made, minced flesh of fresh herring, with and without various antioxidants, being frozen and stored in glass jars at either −10 or −20° C. The results show that ethyl gallate (tables III to VI), n-propyl gallate (tables IV and V), n-butyl gallate (table IV), hexyl gallate (table VI), ascorbic acid and

TABLE IV. Effect of gallic acid esters and dodecyl thiodipropionate on development of rancidity in minced herring flesh stored at −10° C. Peroxide value (P.V.) determined on steam extracted fat after 107 days' storage and thereafter on chloroform extracted fat.

Treatment	P.V. after days		
	107	176	231
Untreated	30.1	78.0	148
0.025% ethyl gallate	16.1	18.3	47.5
0.05% ethyl gallate	6.7	10.9	25.5
0.025% n-propyl gallate	14.9	23.5	52.5
0.05% n-propyl gallate	7.4	10.4	29.1
0.025% n-butyl gallate	15.7	19.9	48.8
0.05% n-butyl gallate	9.0	13.0	22.9
0.05% dodecyl thiodipropionate	26.4	54.5	75.5

cysteine hydrochloride (table V) all caused a marked inhibition in the rate of development of rancidity. On the other hand dodecyl thiodipropionate (tables IV and V), thiourea (table V) and ethanol ammonium gallate (table VI) had either little or no protective action, or, in the case of thiourea, exhibited a prooxidant effect. Fat oxidation in split fresh herring was retarded by dipping them in 0.2% solutions of either ethyl gallate or ethanol ammonium gallate

TABLE V. Effect of six different antioxidants used in 0.05% concentration on development of rancidity in minced herring flesh stored at −20°C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	n-Propyl gallate	Ascorbic acid	Thiourea	Cysteine hydrochloride	Dodecyl thiodipropionate
57	1.9	0.1	0.5	0.5	1.9	0.5	1.5
100	3.3	1.1	1.6	1.1	15.7	2.4	4.3
150	5.5	2.0	2.9	2.6	42.5	3.7	5.2
310	32.9	8.0	9.9	16.0	97.8	24.4	31.1

TABLE VI. Effect of ethyl gallate, hexyl gallate and ethanol ammonium gallate in 0.02% concentration on development of rancidity in minced herring flesh stored at −10° C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	Hexyl gallate	Ethanol ammonium gallate
15	6.9	1.4	2.3	6.9
35	11.1	8.3	11.0	13.8
70	45.3	30.5	57.1	77.9
91	61.3	56.0	56.5	71.1

(table VII). Since ethanol ammonium gallate is very soluble in water it is assumed that a much greater concentration was attained in the split herring than the 0.02% which was without effect in the experiment with minced herring flesh (table VI).

TABLE VII. Effect of ethyl gallate and ethanol ammonium gallate on the development of rancidity in split herring stored at −20° C., as determined by peroxide values.

Days stored	Untreated	Dipped in 0.2% ethyl gallate	Dipped in 0.2% ethanol ammonium gallate
16	0.5	0	0.3
49	1.9	0.4	0
98	5.1	2.2	4.0

Spring Salmon. Minced flesh of fresh red spring salmon, with and without added antioxidants, was frozen and stored in glass jars at different temperatures. It was found that ascorbic acid, ethyl gallate, n-propyl gallate and cysteine hydrochloride all retarded development of rancidity (tables VIII and IX), dodecyl thiodipropionate had no protective value and thiourea at first retarded and subsequently accelerated fat oxidation. In these experiments ascorbic acid and the gallates strongly retarded bleaching of the red astacin pigments of salmon flesh, while cysteine hydrochloride did not have this effect. The rate of fat oxidation bore an inverse relationship to the storage temperature (table IX).

TABLE VIII. Effect of six different antioxidants used in 0.05% concentration on development of rancidity in red spring salmon flesh stored at −20° C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	n-Propyl gallate	Ascorbic acid	Thiourea	Cysteine hydrochloride	Dodecyl thiodipropionate
55	1.0	0	0	0	0.3	0.2	1.1
103	2.2	0.2	0.3	0.4	0.2	0.7	1.9
176	5.6	2.0	1.5	0.2	5.0	1.3	4.6
260	9.9	4.7	4.3	0.4	18.4	4.0	11.1
355	14.3	10.9	8.0	0.6	64.4	7.3	12.3

TABLE IX. Effect of ethyl and n-propyl gallate on development of rancidity in minced red spring salmon flesh stored at −10, −20 and −28° C., as determined by peroxide values.

Days stored	−10° C.			−20° C.			−28° C.		
	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate
32	3.1	0.4	0.2	0.5	0	0	...	...	...
64	8.1	0.5	1.5	0.2	0	0	0.5	0.2	0.2
96	21.8	7.9	7.1	2.7	0.6	0.3	0.6	0	0
140	15.6	5.2	4.8	2.8	0.4	0.4	1.0	0.1	0
212	25.4	6.6	6.4	6.8	2.2	2.4	2.7	0.8	0.9
361	33.7	7.3	7.3	13.7	5.2	6.8	4.1	1.5	1.8

The influence of heating (cooking) untreated and treated minced red spring salmon flesh, which had been stored at −20° C., on the peroxide value of extracted fat was determined. Samples were "cooked" by placing the jars of defrosted fish flesh in warm water, covering them, and exposing them for 10 minutes to boiling water. The types of treatment and results of analysis of uncooked and cooked samples are given in table X. The results showed that lower peroxide values were obtained in the heated flesh irrespective of the method of pre-treatment, and that while ethyl gallate exerted marked antioxidant activity, $NaNO_2$ acted as a pro-oxidant.

TABLE X. Effect of cooking on the peroxide values of the fat of untreated and treated red spring salmon flesh stored at −20° C.

Days stored	Untreated		0.02% ethyl gallate		0.02% $NaNO_2$	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0	0	0	0	0	0	0
39	2.0	0.9	0.1	0	5.0	2.5
79	5.5	2.7	1.1	0.6	9.2	3.8
128	12.4	6.7	3.8	3.0	15.9	6.3
186	17.0	13.5	6.6	4.9	15.6	7.6
266	20.3	10.8	13.3	8.3	16.5	13.6

Twenty-four fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from two 8 to 9-pound (3.6 to 4.1-kg.) red spring salmon, dipped in 10% NaCl solution, and drained. Eleven fillets were left untreated, the remainder being dipped in 0.25% ethyl gallate solution. Analysis of the pooled minced flesh of two of the treated fillets showed that it contained 0.0096% ethyl gallate. Treated and untreated fillets were stored at both −10 and −28° C. The results of the experiment (table XI) show that rancidity developed much more rapidly at the higher storage temperature, and that at both storage temperatures ethyl gallate delayed fat oxidation considerably. The rather irregular increase in peroxide values obtained during storage was probably because fat oxidation does not

TABLE XI. Effect of ethyl gallate on development of rancidity in red spring salmon fillets stored at −10 and −28° C., as determined by peroxide values (P.V.).

Days stored	−10° C.		−28° C.	
	Controls	Ethyl gallate	Controls	Ethyl gallate
* 84	4.3	1.5	0.2	0
*116	5.4	2.1	1.3	0.4
*149	17.4	5.5	1.4	0.9
†196	13.3	5.0	5.1	0.3
†226	26.8	10.2	1.4	0.5
†317	....	....	4.3	0

*P.V. determined on steam extracted fat.
†P.V. determined on chloroform extracted fat.

proceed at the same rate in different fish or parts of the same fish, due to such possible differences as variations in fat content, area of exposed fat and amount of fat oxidizing enzyme (*vide infra*) present. Also the treated fillets may have had somewhat different concentrates of ethyl gallate.

Pink Salmon. The minced flesh from two 4-lb. (1.8-kg.) pink salmon (*Oncorhynchus gorbuscha*) was treated in different ways and then samples were stored at −10, −20 and −28° C. The methods of treatment employed and the experimental results are given in table XII. The results showed that the

TABLE XII. Effect of ethyl gallate, n-propyl gallate, ascorbic acid, sodium nitrite and sodium chloride on development of rancidity in minced pink salmon flesh.

Treatment	Peroxide value after days											
	−10° C.				−20° C.				−28° C.			
	45	90	176	215	45	90	176	215	45	90	176	215
Untreated	5.8	12.4	33.8	38.8	0.8	2.3	10.8	15.1	0.2	1.0	2.0	3.5
0.02% ethyl gallate	0.3	1.7	12.5	13.4	0.2	0.7	2.0	4.2	0	0.2	0.6	0.8
0.02% n-propyl gallate					0.1	0.8	3.2	5.0				
1.0% NaCl					5.1	12.7	24.5	33.4				
1.0% NaCl + 0.02% ethyl gallate					0.7	2.3	6.7	11.8				
1.0% NaCl + 0.02% n-propyl gallate					0.5	2.5	14.3	17.1				
1.0% NaCl + 0.02% $NaNO_2$					6.5	13.3	29.7	31.8				
0.02% $NaNO_2$					1.6	7.1	20.5	21.4				
0.05% ascorbic acid					0.2	0.5	0.3	1.2				

rate of fat oxidation was considerably reduced by lowering the storage temperature, and that ethyl gallate inhibited development of rancidity to about the same extent at each of the three storage temperatures. At −20° C. ethyl and n-propyl gallate protected the fish to about the same extent, and both considerably delayed the onset of rancidity in fish flesh treated with NaCl, which itself accelerated fat oxidation. Sodium nitrite exerted a pro-oxidation effect, and a mixture of $NaNO_2$ and NaCl promoted fat oxidation to a somewhat greater extent than did either alone. Ascorbic acid was a more effective antioxidant than were ethyl and n-propyl gallates.

Fillets were prepared from five 4-lb. (1.8-kg.) pink salmon, each fillet being cut in two pieces of approximately equal size. Four of the resulting small fillets (one from each fish) were dipped into solutions the composition of which are given in table XIII. The amount of gallate present was determined in one fillet from each treatment, and the remainder were stored at −10° C. The results (table XIII) show that ethyl and n-propyl gallate (average content 0.0143%) when thus incorporated into the flesh retarded fat oxidation considerably, and that initial buffering to pH 6.2 of the gallate solutions used did not appreciably affect their antioxidant power.

Chum Salmon. The compounds listed in table XIV were incorporated into the minced flesh of a 7.5-lb. (3.4-kg.) chum salmon (*Oncorhynchus keta*), and

the resulting samples were stored at −10° C. It will be seen from the results given in table XIV that ethyl gallate considerably retarded the onset of rancidity, and that ascorbic acid was not very effective in the concentration used. Neither citric nor tartaric acid retarded fat oxidation.

TABLE XIII. Effect of ethyl and n-propyl gallate on development of rancidity in pink salmon fillets stored at −10° C., as determined by peroxide values.

Days stored	Untreated fillets	*Dipped in 0.5% †ethyl gallate	*Dipped in 0.5% †ethyl gallate + 0.05 M sodium phosphate buffer pH 6.2	*Dipped in 0.5% †n-propyl gallate	*Dipped in 0.5% †n-propyl gallate + 0.05 M sodium phosphate buffer pH 6.2
43	3.5	0.5	0.5	0.2	0.3
67	8.6	2.5	3.0	2.5	1.4
97	10.4	5.1	4.5	3.3	2.8

*Final pH of buffered solutions about 6.3; initial pH of unbuffered gallate solutions about 4.5, and final pH about 6.2.

†Gallate content of fillets: Untreated fillets, none; dipped in 0.5% ethyl gallate, 0.014%; dipped in buffered 0.5% ethyl gallate, 0.0137%; dipped in 0.5% propyl gallate, 0.0104%; dipped in buffered 0.5% propyl gallate, 0.019% (Average = 0.0143%).

TABLE XIV. Effect of ethyl gallate, ascorbic acid, tartaric acid and citric acid on development of rancidity in minced chum salmon flesh stored at −10° C., as determined by peroxide values.

Days stored	Untreated	0.02% ethyl gallate*	0.02% ascorbic acid	0.02% citric acid	0.02% tartaric acid
52	27.9	7.6	9.15	29.5	21.6
101	31.3	14.8	25.2	39.2	38.0
132	58.0	23.6	47.7	74.5	60.3

* "Progallin A."

TABLE XV. Effect of n-propyl gallate, ascorbic acid and sodium chloride on development of rancidity in chum salmon fillets stored at −10° C., as determined by peroxide values.

Days stored	Untreated fillets	Dipped in 0.5% n-propyl gallate	Dipped in 20% NaCl	Dipped in 20% NaCl containing 0.5% n-propyl gallate*	Dipped in 0.5% ascorbic acid (pH 6.0)	Dipped in 0.5% ascorbic acid dissolved in 20% NaCl (pH 6.0)**
53	9.4	1.5	20.5	2.0	14.0	19.0
103	20.0	4.9	124.0	14.0	24.2	31.0
165	50.3	11.1	105.0	25.0	31.0	42.0

*The NaCl caused the gallate to crystallize from the solution at 20° C., so that this solution had to be used at 40° C.

**The pH of the ascorbic acid solutions was adjusted to 6.0 with 1.0 N. NaOH immediately prior to use.

Eighteen fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from three 8-lb. (3.6-kg.) chum salmon. Three fillets were subjected to each of the treat-

ments recorded in table XV, and all were then stored at −10° C. The results (table XV) showed that NaCl treatment accelerated the onset of rancidity, and that n-propyl gallate treatment inhibited it. Immersion in ascorbic acid solutions alone did not hinder fat oxidation, but fillets which were dipped in NaCl solutions containing ascorbic acid did not become rancid as rapidly as those treated in NaCl solutions alone. It is suggested that NaCl facilitated the penetration of ascorbic acid into the flesh. Recent experiments (unpublished) have shown that immersion of fillets for at least 5 minutes in 1% ascorbic acid solution is necessary if a concentration of approximately 0.05% of the acid in the flesh is to be attained.

TABLE XVI. Effect of ethyl gallate, n-propyl gallate and sodium nitrite on development of rancidity in minced coho salmon flesh stored at −10 and −20° C., as determined by peroxide values.

Days stored	−10° C.				−20° C.			
	Untreated	0.02% ethyl gallate	*0.02% n-propyl gallate	0.02% sodium nitrite	Untreated	0.02% ethyl gallate	*0.02% n-propyl gallate	0.02% sodium nitrite
42	6.2	0.9	0.8	16.2	....	....	....	....
64	12.4	6.5	4.5	14.7	2.8	0.6	0.4	8.9
109	31.2	10.6	5.8	31.4	6.3	1.8	1.1	11.6
196	43.5	20.9	14.9	42.0	24.8	11.2	10.1	27.2
263	57.3	14.5	13.3	46.5	32.1	21.4	19.4	23.2
289	....	....	....	....	22.6	34.2	23.1	42.1

*Progallin P.

TABLE XVII. Effect of sodium chloride, ethyl gallate and ascorbic acid on development of rancidity in flesh of black cod stored at −10° C., as determined by peroxide values.

Days stored	Untreated	0.02% ethyl gallate	1.0% NaCl	0.02% ethyl gallate + 1.0% NaCl	0.05% ascorbic acid
35	0.7	0.5	0.5	0.4	0.1
69	2.1	2.5	3.0	3.2	0.1
108	4.9	4.9	5.7	8.1	0.3
158	3.3	5.1	6.8	6.3	0.4

Coho Salmon. The flesh from four 4-lb. (1.8-kg.) coho salmon ("bluebacks") was minced, and the flesh subjected to different treatments (table XVI). The results obtained on storing untreated and treated samples at −10 and −20° C. are recorded in table XVI. It will be seen that fat oxidation proceeded very much more rapidly at −10 than at −20° C. At both these temperatures 0.02% of ethyl or n-propyl gallate considerably delayed the onset of rancidity while 0.02% $NaNO_2$ exerted a pro-oxidant effect.

Black Cod. Minced flesh was prepared from a 16-lb. (7.25-kg.) black cod (*Anaplopoma fimbria*), sodium chloride, ethyl gallate and ascorbic acid were incorporated, and the samples stored at −10° C. The methods of treatment

and results are recorded in table XVII. It will be seen that fat oxidation was only slightly accelerated by sodium chloride, and that ethyl gallate did not retard this oxidation in either untreated or NaCl-treated flesh. Ascorbic acid strongly retarded development of rancidity.

Stabilization of Colour

The development of rancidity in red salmon is accompanied by a superficial bleaching of the red astacin pigments, which is particularly evident in regions where the flesh is exposed to atmospheric oxygen. Heating the flesh also has a marked bleaching effect on colour. The following experiment was made in order to determine the effect of ethyl and n-propyl gallate and of $NaNO_2$ on the colour of salmon flesh after heating it, and also during prolonged storage in the frozen state. Aluminum dishes 5.5 cm. in diameter and 1.3 cm. deep were packed with minced coho or red spring salmon flesh which had received the treatments recorded in tables XVIII and XIX. Some of the samples were placed in covered

Table XVIII. Effect of ethyl gallate, n-propyl gallate and $NaNO_2$ on the red and yellow colour* of the surface of raw and heated coho salmon flesh, and of the raw flesh after freezing and storage at −20° C.

Treatment	Raw		Heated		Period stored at −20° C. (days)									
					64		141		219		303		303†	
	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
Untreated.....	12	6	6	3.5	5.5	3.5	5.5	3	3.5	3	2.5	3	11	6
0.02% ethyl gallate.....	12	6	6	3.5	6	3.5	6.0	3	6	3	6	3.5	11	6
0.02% n-propyl gallate.....	11.5	6	6	3.5	6	3.5	6.5	3	6.5	3	6.5	3.5	11	6
0.02% $NaNO_2$...	11.5	6	6.5	3.5	6.5	4.0	4.5	3	3	3	2.5	3	11.5	6
0.02% ethyl gallate + 0.002% $NaNO_2$	11.5	6	6.5	3.5	6.5	4.0	6.5	3	6	3	5.5	3.5	10.5	6

*In tables XVIII and XIX the colour is given in red (R) and yellow (Y) Lovibond units.

†The figures in this column are for the surface obtained after cutting the samples in half and thawing the exposed area. They show that the colour loss was largely superficial.

pyrex glass petri dishes, steamed for 20 minutes at 100° C., and then cooled. Others were stored in similar containers at −20° C. The surface colour of the raw and heated flesh, and of the stored samples, was measured in red and yellow Lovibond units using an Armstrong colorimeter (Charnley 1936). The results (tables XVIII and XIX) show that, in general, the different treatments affected coho and red spring salmon similarly. Heating occasioned considerable loss in both red and yellow colour of untreated flesh. The gallates exerted no important effect on the colour of either raw or heated flesh. Sodium nitrite treatment alone, or in combination with ethyl gallate, slightly lowered the red colour of raw flesh but intensified that of heated flesh. Freezing caused an initial marked

TABLE XIX. Effect of ethyl gallate, n-propyl gallate and $NaNO_2$ on the red and yellow colour of the surface of raw and heated red spring salmon flesh, and of the raw flesh after freezing and storage at −20° C.

Treatment	Raw		Heated		Period stored at −20° C. (days)									
					62		131		209		294		294*	
	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
Untreated.....	11	6.5	6	3.5	5	3	4	2.5	3.5	2.5	3	3	5.5	4
0.02% ethyl gallate.....	11	6.5	6	3.5	6	3	6	2.5	5.5	2.5	5.5	3.5	6.5	4
0.02% n-propyl gallate.....	11	6.5	6	3.5	6	3	6	2.5	5.5	2.5	6	4	7	4
0.02% $NaNO_2$...	10.5	6.5	6.5	3.5	5	3	3.5	2	2.5	2.5	2.5	3	9	4
0.02% ethyl gallate + 0.002% $NaNO_2$	10.5	6.5	6.5	3.5	6	3.5	4	2	5	2.5	5	3.5	6.5	4

*The figures in this column are for the surface obtained after cutting the samples in half and thawing the exposed area. The results in this case differ from those obtained with coho salmon (table XVIII) in that there was considerable loss of red colour in the sub-surface flesh, though this loss was less marked in treated samples than it was in the control.

loss in both red and yellow colour, and this may have been due to surface drying or some other effect, because freshly cut thawed surfaces of the coho salmon after 303 days' storage at −20° C. gave red and yellow colour readings nearly as great as those obtained initially in the raw flesh. In the stored frozen samples there was a marked progressive diminution in the red colour, and this loss was more rapid in flesh treated with $NaNO_2$. Both ethyl and n-propyl gallates prevented, or very strongly retarded, loss of red colour in the stored samples, and ethyl gallate prevented loss in colour in flesh treated with 0.002% $NaNO_2$. The yellow colour of the cold-stored fish samples differed slightly, and at that rather irregularly, in the different samples, and there is no definite indication that the small observed fluctuations were due to any of the treatments used. That the loss of red colour in the frozen coho salmon was largely superficial was apparent from the colour readings obtained in the thawed sub-surface flesh. In the red spring salmon this difference was not as marked, for the red pigments of the sub-surface flesh had been noticeably bleached.

DISCUSSION

Banks (1937) provided experimental evidence which proved that the rancidity developing in cold-stored herring was largely enzymic in origin, and that the lipoxidase enzyme concerned was activated by pure sodium chloride. He also showed that oxidation of the fat in frozen herring proceeded fairly slowly at −28° C., and much more rapidly at higher storage temperatures (1938). A preliminary storage at fairly high temperatures (e.g. −2.5 or −10° C.) was found to partially inactivate the lipoxidase enzyme so that on subsequent storage at lower temperatures fat oxidation in herring thus treated was greatly retarded (Banks 1939). It would seem probable that this inactivation may be linked up

with denaturation of a protein group of the enzyme concerned at high storage temperatures.

The results obtained in the present work with frozen coho, red spring and pink salmon, showed that rancidity developed much more rapidly at high than at low storage temperatures. It was also found that the onset of rancidity in cold-stored herring, black cod and salmon was hastened by treatment with pure sodium chloride. These findings confirm and extend those of Banks. However, in this connection it must be noted that recent work (Tarr 1946, and unpublished) has shown that sodium chloride treatment does not invariably accelerate fat peroxide formation, especially with autumn-caught fish, and also that the rate of peroxide formation may vary enormously with different lots of the same variety of fish stored at the same temperature. There are indications that fat oxidation proceeds very rapidly in frozen flesh prepared from "feedy" herring, and it is possible that lipoxidase enzymes from the feed gain access to the herring flesh.

Sodium nitrite in 0.02% concentration invariably accelerated the onset of rancidity in frozen fish, but had the reverse action in unfrozen fish, possibly due to its bacteriostatic action. Lea (1936) found that at pH values below 5.0, 0.03% of sodium nitrite was a powerful pro-oxidant, the activity of which was not affected by antioxidants which he studied. It would seem that at the usual pH of fresh fish flesh (6.0 to 6.8) nitrites are also able to act as pro-oxidants.

The experiments described showed that, except in the case of black cod, fairly low concentrations (e.g. 0.02 or 0.05%) of the lower alkyl esters of gallic acid (particularly ethyl and n-propyl gallates) markedly retarded fat oxidation in frozen fish, and largely prevented bleaching of the red astacin pigments of salmon. When used in similar (per cent) concentration the above esters appeared to be about equally effective. On the other hand the salt, ethanol ammonium gallate, was ineffective in 0.02% concentration. Whether or not the gallates only act directly as antioxidants, or also indirectly by partially inactivating the lipoxidase enzyme, is not certain. Gallic acid and its ester, the use of which as antioxidants in foods has been patented by Sabalitschka and Boehm (1941, 1942), have been found to be excellent antioxidants for whole dried milk (Findlay, Smith and Lea 1945), dried meat (Lea 1944a) and certain edible fats (Golumbic and Mattill 1942; Lea 1944b; Higgins and Black 1944). Moreover, the lower esters, when used in 0.02% concentration, do not affect the taste of treated products (Lea 1944a and b). The toxicity of ethyl or n-propyl gallates toward white mice and rats is extremely low (Boehm and Williams 1943; Hilditch 1944).

Ascorbic acid in 0.05% concentration was found to be a very good antioxidant for minced flesh of herring, red spring salmon, pink salmon and black cod. In one experiment using chum salmon 0.02% of this compound retarded fat oxidation only very slightly. Experiments which are still in progress indicate that immersion of fillets for about 5 minutes in ice-cold ascorbic acid solution (e.g. 1%) is required in order to attain a sufficiently high concentration to retard development of rancidity in treated flesh effectively. Ascorbic acid has been used successfully as an antioxidant for whole dried powdered milk (Findlay *et al.* 1945), for fats (Mattil, Filer and Longenecker 1944) and for certain other

foods (Gray and Stone 1939). Younga, Esselen and Fellers (1944) have shown that d-isoascorbic acid also possesses marked antioxidant activity.

The results obtained with thiourea, which has been shown to retard development of rancidity of fats in aqueous systems (György, Stiller and Williamson 1943) were not encouraging. In one experiment it inhibited the onset of rancidity slightly during early stages of storage, but subsequently acted as pro-oxidant, while in a second experiment it merely acted as pro-oxidant. Williamson (1944), working with powdered milk, found that thiourea was quite a good antioxidant during the early stages of storage but eventually exerted marked pro-oxidant activity in many cases. Cysteine hydrochloride markedly retarded fat oxidation, but it did not prevent bleaching of the red pigments of salmon flesh. Dodeycyl thiodipropionate did not protect the fats of fish flesh against oxidation.

There is little doubt that development of rancidity in stored frozen fish is one of the principal factors leading to its deterioration. Undesirable changes such as the appearance of a "salt fish" flavour in white fish of low fat content, of definite organoleptic rancidity in the flesh of fatty fish, and of bleaching of natural red pigments and "rusting" of exuded fat must all be included under this heading. The rate of development of well-defined organoleptic rancidity in cold-stored fish varies with different species, and is probably influenced by a number of factors such as changes in fat content, degree of unsaturation of the fats, and amount of lipoxidase enzyme in the tissue. Though a quantitative determination of the peroxide value of the fat of frozen fish might give a definite indication that the fish is or is not rancid, it is unlikely that such a test can ever be used to divide fish flesh into grades. Thus organoleptic rancidity has first been detected in kippers when peroxide values as different as 2.2 and 14.3 have been obtained (Banks and Reay 1938; Banks, Cutting and Reay 1939). In the present work it was found that in certain samples, and especially in those stored at $-10°$ C., there was initially a rapid increase in the peroxide value, but that this frequently became either stationary or only rose slowly. This may have been due to inability of oxygen to penetrate to the sub-surface flesh, or to a breakdown of the fat peroxides, or to both. In any case it indicates one of the difficulties which might arise in attempts to apply a determination of the peroxide value of the fats as a criterion of the quality of frozen fish.

SUMMARY

The effect of a number of chemical antioxidants, alone or in conjunction with NaCl and $NaNO_2$, on the development of rancidity in the indigenous fat of salmon, herring and black cod flesh stored at different temperatures was investigated.

At 0° C. ethyl gallate, sodium gallate and $NaNO_2$ retarded both fat oxidation and bacterial increase, while NaCl acted as a pro-oxidant.

In frozen flesh rancidity developed very slowly at $-28°$ C., and somewhat more rapidly at $-20°$ C.; while storage for a month or less at $-10°$ C. usually led to marked fat oxidation. Ethyl, n-propyl, n-butyl and hexyl gallates when used in from about 0.01 to 0.05% concentration in different experiments considerably retarded the onset of rancidity, while sodium gallate and ethanol

ammonium gallate (0.02%) were ineffective. Ascorbic acid in 0.05% concentration in minced flesh proved a very effective antioxidant, but in one experiment 0.02% was relatively ineffective. Cysteine hydrochloride retarded fat oxidation but did not prevent bleaching of the red astacin pigments of salmon flesh. Dodecyl thiodipropionate, thiourea and citric and tartaric acid were not found suitable as antioxidants for fish flesh.

The red and yellow pigments of coho and red spring salmon flesh faded considerably on heating and, superficially, on freezing. The marked superficial fading of the red colour of frozen coho and red spring salmon flesh during storage was largely prevented by pre-treatment with 0.02% of ethyl or n-propyl gallate, but was accelerated by $NaNO_2$ treatment.

Both NaCl and $NaNO_2$ accelerated oxidation of fat in frozen fish flesh.

ACKNOWLEDGMENTS

I wish to thank Mrs. F. M. Kwong for her skilled technical assistance during the course of this work. To the Heyden Chemical Corporation, The Silmo Chemical Corporation, Canadian Industries, Ltd., Merck and Co. Ltd., Nipa Laboratories, Ltd. and Dr. C. H. Lea I am indebted for supplies of certain of the antioxidants used in these experiments. The colorimeter was kindly loaned by the Armstrong Laboratories, Vancouver.

REFERENCES

ANON. *Ann. Rep. Coun. Sci. Ind. Res. Australia*, **14**, 1-102, 1940.
BAHR, O., AND O. WILLIE. *Brit. Patent No.* 386, 482, 1933.
BANKS, A. *J. Soc. Chem. Ind.*, **56**, 13T-15T, 1937.
J. Soc. Chem. Ind., **57**, 124-128, 1938.
Gr. Brit. Food Inv. Bd. Ann. Rep., **1938**, 106-112, 1939.
BANKS, A., C. L. CUTTING AND G. A. REAY. *Gr. Brit. Food Inv. Bd. Ann. Rep.*, **1938**, 98-102, 1939.
BANKS, A., AND G. A. REAY. *Gr. Brit. Food Inv. Bd. Ann. Rep.*, **1937**, 81-84, 1938.
BEILSTEIN, K. F. Handbuch der Organischen Chemie. 4th ed., **10**, 1-1124, 1927.
BLACK, H. C. Quartermaster Corps Manual QMC, **17-7**, 35-39, Washington, 1945.
BOEHM, E., AND R. WILLIAMS. *Quart. J. Pharm. Pharmacol.*, **16**, 232-243, 1943.
CHARNLEY, F. *Biol. Bd. Can. Prog. Rep. Pac.*, **29**, 12-16, 1936.
FINDLAY, J. D., J. A. B. SMITH AND C. H. LEA. *J. Dairy Res.*, **14**, 165-175, 1945.
GOLUMBIC, C., AND H. A. MATTILL. *Oil and Soap*, **19**, 144-145, 1942.
GRAY, P. P., AND I. STONE. *Food Industr.*, **11**, 626-628, 1939.
GYORGY, P., E. T. STILLER AND M. B. WILLIAMSON. *Science*, **98**, 518-520, 1943.
HIGGENS, J. W., AND H. C. BLACK. *Oil and Soap*, **21**, 277-279, 1944.
HILDITCH, T. P. *Chem. and Ind.*, **1944** 67-71, 1944.
JENSEN, L. B. Microbiology of meats. 1-389, Garrard Press, Champaign, Ill., 1945.
LEA, C. H. *J. Soc. Chem. Ind.*, **55**, 293T-302T, 1936.
Rancidity in edible fats. *Gr. Brit. Food Inv. Bd. Spec. Rep.* **46**, 1-230, 1938.
J. Soc. Chem. Ind., **63**, 55-57, 1944a.
J. Soc. Chem. Ind., **63**, 107-112, 1944b.
J. Soc. Chem. Ind., **64**, 106-109, 1945.
LEMON, J. M., M. E. STANSBY AND C. E. SWIFT. *Food*, **6**, 441-443, 1937a.
Food Industr., **9**, 576-577, 583, 1937b.
MATTIL, K. F., AND L. J. FILER. *Ind. Eng. Chem. (Analyt.)*, **16**, 427-429, 1944.
MATTIL, K. F., L. J. FILER AND H. E. LONGENECKER, *Oil and Soap*, **21**, 160-161, 1944.

PETERS, F. N., AND S. MUSHER. *Ind. Eng. Chem.*, **29**, 146-151, 1937.
PLATNER, W. S. *Ind. Eng. Chem.* (*Analyt.*), **16**, 369, 1944.
SABALITSCHKA, T., AND E. BOEHM. *U.S. Patent No.* 2, 255, 191, 1941.
Brit. Patent No. 542, 833, 1942.
SILVER, R. E. *Food Industr.*, **17**, 1454-1456 and 1596-1600, 1945.
SMITH, F. H., D. E. BRADY AND R. E. COMSTOCK. *Ind. Eng. Chem.*, **37**, 1206-1209, 1945.
STANSBY, M. E., AND J. M. LEMON. *U.S. Fish Wildlife Serv., Res. Rep.*, **1**, 1-46, 1941.
STANSBY, M. E., AND R. W. HARRISON. *U.S. Fish Wildlife Serv., Spec. Sci. Rep.*, **15**, 1-25, 1942.
STUFFINS, C. B., AND H. WEATHERALL. *Analyst*, **70**, 403-409, 1945.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **5**, 265-275, 1941.
J. Fish. Res. Bd. Can., **6**, 74-89, 1942.
J. Fish. Res. Bd. Can., **6**, 119-128, 1943.
Nature, **154**, 824-826, 1944a.
J. Canad. Dietetic Assn., **6**, 71-76, 1944b.
Fish. Res. Bd. Can. Prog. Rep. Pac., **64**, 57-61, 1945.
Fish. Res. Bd. Can. Prog. Rep. Pac., **66**, 17-20, 1946.
WILLIAMSON, M. B. *Food Res.*, **9**, 298-303, 1944.
YOUNGA, F. J., W. B. ESSELEN AND C. R. FELLERS. *Food Res.*, **9**, 188-196, 1944.